Synthesis of deuterium-labeled crizotinib , a potent and selective dual inhibitor of mesenchymal-epithelial transition factor (c-MET) kinase and anaplastic lymphoma kinase (ALK)

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Abstract: To more accurately and rapidly achieve quantitative detection of clinical crizotinib samples, stable isotope labeled crizotinib was required as an internal standard. We have developed a method to prepare racemic [D_9]crizotinib using a base-catalyzed H/D exchange of both nitro compound **2** and the acetophenone compound **6** with D_2O and $NaBD_4$ reduction of **7** as the key steps to introduce the nine deuterium atoms. Starting with 4-hydroxypiperidine, 14 step-synthesis furnished the desired racemic [D_9]crizotinib **18**. The deuterium-labeled compound **18** with the chemical purity of 99.62% was applicable for use as internal standards in the drug clinical study.

Key words: Deuteration; H-D exchange; Internal Standard; Crizotinib; c-MET; ALK

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Introduction

In 2011, crizotinib (brand name Xalkori, Fig.**1**) became the first ALK inhibitor approved by the USA Food and Drug Administration (FDA) as a first-line treatment for ALK-positive lung cancer patients.[1-4]

Stable isotope-labeled compounds have been proven to be ideal internal standards for use in a human absorption, distribution, metabolism, and excretion (ADME) studies.[5] Among all of the internal standard compounds developed, deuterium-labeled compounds are well known to be the first choice for such applications due to their similarity of physical and chemical properties with original compounds and easy availability in terms of deuterium source and synthetic approach.[6]

To our knowledge, there were few procedures described in literature for the preparation of deuterium-labeled crizotinib.[7-8] The known approaches either need expensive deuterium sources, such as $CDCl_3$ and DCOD, or only can offer one or two deuterium labeled crizotinib. For crizotinib, the abundance of the M+5 (454 amu) isotopologues comes to 1.5%, considering the natural isotope distribution of ^{35}Cl/ ^{37}Cl and ^{12}C/^{13}C. Therefore, a minimum of six deuterated sites for the internal standard appeared to be sufficient to prevent unfavorable interferences between signals of non-deuterated and deuterated crizotinib in a quantitative bioanalytical liquid chromatography/tandem mass spectrometry (LC/MS/MS) assay.

Considering the structure and possible synthetic approach of crizotinib (Fig. **1**), a 14-step labeling process (Scheme **1** , **2** & **3**) was developed to prepare the desired deuterium-labeled R/S-crizotinib **18**.

RESULTS AND DISCUSSION

Methods for the synthesis of crizotinib have been reported in the literature,[3, 9-10] but the key approach is basically the same. According to the existing synthetic route of the original drug, we designed the synthetic routes shown in Scheme **1**, **2** & **3** to successfully prepare the deuterated crizotinib **18**.

To activate the ortho-hydrogen of the nitrogen atom, nitroso group was first introduced into the nitrogen atom of 4-hydroxypiperidine **1** to form nitroso compound **2** in 78% yield, and then four deuterium atoms can be easily introduced to give compound **3** after the hydrogen-deuterium (H-D) exchange with D_2O under basic (NaOD) conditions.[11] Removal of nitroso group by using aluminum-nickel (Al-Ni) alloy under alkaline condition afforded 4-[D_4]hydroxypiperidine **4**. Without isolation of **4,** direct *N*-acylation of **4** with di-tert-butyl dicarbonate furnished **5** with a protection group in overall 25% yield (3 steps). From **2**, **5** was prepared in one pot without the isolation of intermediates **3** & **4** since H-D exchange, (Al-Ni) alloy reduction and Boc protection reactions used the same basic solution as a solvent.

Although Hesk[12] et al. has described the three-step approach toward the synthesis of **5,** it needed expensive dueterated formaldehyde and lacked analytical data. Our new approch to **5** uses much cheaper and more readily availabe D_2O as only deuterium source to offer >95% deuterium enrichment.

Furthermore, four deuterium atoms were introduced into **7** after the H-D exchange in a mixed solution of D_2O and 1,4-dioxane under a basic (NaOD) condition in a 92% yield.[13-14] Unlike the literature,[7] our NaOD promoted H-D exchange reaction allowed not only deutrium atoms to be incorpoarted into α methyl group of the ketone **7**, but also into 3 position of phenyl ring. To avoid the back-exchange of **7** with the non-deuterated agents and solvents, deuterated sodium boron hydride ($NaBD_4$) in a mixed solution of MeOD and THF was applied to reduce the labeled ketone **7** to racemic **8** in a 82% yield. Based on HNMR of **8**, it was observed that the base-catalyzed D-H exchange of **6** with D_2O gave >95% D at 2 position and >93% D at 3' position, respectively, and $NaBD_4$ reduction of the ketone **7** offered 98% D enrichment

(Scheme 2). Mitsunobu reaction of **8** with **9** provided **10** in 42.8% yield, and then **10** was further reduced with zinc powder in acetic acid under reflux to **11**. Bromination of **11** with NBS in acetonitrile produced **12** in 78% yield.

With the key deuterium-labeled intermediates **5** & **12** in hand, deuterated crizotinib was successfully prepared via a 5-step synthetic sequence starting from **5** as depicted in **Scheme 3**. The *N*-mesylation of **5** with methane sulfonyl chloride under a basic (Et_3N) condition provided **13** in 86% yield. *N*-Alkylation of **13** with 3-iodopyrazole **14** generated iodo compound **15** in 70.7% yield. Palladium-catalyzed coupling reaction of **15** with bis(pinacilato)diboron gave the boronic ester derivative **16** in 62.9% yield. Suzuki cross-coupling reaction of two labeled intermediates **12** & **16** offered boc-protected crizotinib **17** that was de-protected with 5% HCl methanol solution to generate R/S-[D_9]crizotinib **18** in a fair yield. It was not necessary to separated R/S isomers since the mixture of two enantiomers as an internal standard (Figure 2) does not affect the accuracy in a quantitative bioanalytical LC/MS/MS assay.

Conclusion

The key points of our deuterium-labeling synthetic approach to R/S-[D_9]crizotinib **18** are summarized as follows: 1) activating the adjacent hydrogen atom of the piperidine moiety by introducing the nitroso group into the secondary amine of 4-hydroxypiperidine, which make the nitroso compound easily exchanged with D_2O to incorporate four deuterium atoms into the key intermediate **5**; and 2) carrying out H-D exchange of acetophenone **6** with D_2O followed by reduction of carbonyl with $NaBD_4$, which allows five deuterium atoms introduced into another intermediate **8**. Although the entire synthetic route is long (14 steps), the reactions involved are relatively conventional and several steps do not require post-treatment.

So far, we have developed a practical method for synthesizing deuterium-labeled crizotinib. The total yield of the 14-step synthesis was 6.2%, which provided a much-needed stable isotope internal standard for clinical studies of crizotinib.

Experimental

General

All reagents were purchased from commercial sources and were used as received. Routine monitoring of reactions was performed by thin layer chromatography (TLC) using pre-coated Haiyang GF254 silica gel TLC plates. NMR spectra were recorded on a Bruker AVANCE 500 spectrometer at 500 MHz with tetramethylsilane used as an internal reference. High resolution mass spectra (HRMS) were performed on Agilent Acrrurate-Mass Q-ToF LC/MS 6520 mass spectrometer with electron spray ionization (ESI) mode. Intermediates **1**, **7** & **9** were purchased from Meryer (Shanghai) Co., Ltd. Intermediates **14** were purchased from Energy chemical Ltd. Deuterium agents, D_2O (99.9% D) and $NaBD_4$ (98.5% D) were obtained from Sigma-Aldrich.

1-Nitrosopiperidin-4-ol (2)

A solution of sodium nitrite (20.0 g, 0.29 mol) in water (100 mL) was slowly added at -5 °C to a solution of piperidin-4-ol (**1**, 25.0 g, 0.25 mol) in water (100 mL) and 4.5 N hydrochloric acid (80 mL, 0.36 mol). The resulting mixture was stirred at room temperature overnight and then concentrated and purified by flash column chromatography (PE:EA = 1:5) to afford **2** (25.3 g, 77.7% yield) as a light yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 4.32-4.36 (m, 1H), 4.03-4.08 (m, 2H), 3.88-3.92 (m, 1H), 3.71-3.78 (m, 1H), 3.69-3.70 (m, 1H), 1.94-1.97 (m, 1H), 1.69-1.79 (m, 2H), 1.50-1.54 (m, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 65.36, 46.76, 35.92, 33.84, 32.23.

***tert*-Butyl 4-[2,2,6,6-D_4]hydroxypiperidine-1-carboxylate (5)**

Sodium methoxide (40.0 g, 0.74 mol) was added to a solution of the compound **2** (19.0 g, 0.15 mol) in D_2O (200 mL). The resulting solution was stirred at 100 °C for 24 hours. The solvent was evaporated under reduced pressure to give the residue which was mixed with D_2O (200 mL). The resulting solution was stirred at 100 °C for another 14.5 hours. The reaction solution can be used for next reaction without purification.

Aluminum and nickel alloy (14 g) were added slowly to the above reaction solution (0.15 mol) over a time period of 50 minutes at 80 °C. The resulting reaction mixture was stirred at 80 °C for 30 minutes, and then cooled to room temperature. The mixture was filtered and the filtrate (about 200 mL) can be used directly for next reaction.

Di-*tert*-butyl dicarbonate (36.8 g, 0.17 mol) was added to the above filtrate (200 mL, 0.15 mol) at room temperature. The resulting reaction mixture was stirred at room temperature for 4 hours and then extracted with DCM (200 mL x 2). The combined organic layers were washed with brine, dried with $MgSO_4$ and evaporated to dryness. The crude residue was purified by flash column chromatography to afford **5** (7.64 g, 25.5% yield for 3 steps) as a yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 3.79-3.84 (m, 1H), 2.17 (s, 1H), 1.82 (dd, J_1 = 13.0 Hz, J_2 = 4.0 Hz, 2H), 1.46 (s, 1H), 1.45 (s, 9H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 154.85, 79.51, 67.51, 40.10-41.28 (m, C-D), 33.91, 28.42. HRMS (ESI+, $[M+H]^+$) *m/z*: 206.1681.

1-(2,6-Dichloro-3-fluoro[5-D]phenyl) [1,2,2,2-D_4]ethan-1-ol (8)

Sodium methoxide (0.54 g, 0.01 mol) was added to a solution of the 1-(2,6-dichloro-3-fluorophenyl)ethan-1-one (**6**, 8.32 g, 0.04 mol) in D_2O (100 mL) and 1,4-dioxane (100 mL). The resulting solution was stirred at 100 °C for 6 hours. The solvent was evaporated under reduced pressure to give the residue which was mixed with D_2O (100 mL) and 1,4-dioxane (100 mL). The resulting solution was stirred at 100 °C for another 6 hours. The solvent was evaporated under reduced pressure to dryness to give crude **7** (7.8 g) as a colorless oil.

MeOD (5.0 mL, 123.59 mmol) was slowly added to a mixture of **7** (7.50 g, 35.54 mmol) and boron sodium deuteride (1.76 g, 42.08 mmol) in dry THF (30 mL) at room temperature. The resulting mixture was stirred at 60 °C for 1 hour, and then quenched with H_2O (100 mL) at 0 °C. The mixture was extracted with EtOAc (100 mL x 2). The combined organic layers were dried with $MgSO_4$ and evaporated to dryness. The crude residue was purified by flash column chromatography (PE:EA = 5:1) to afford **8** (6.27 g, 82.4% yield) as a colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.26 (d, J = 4.5 Hz, 1H), 2.83 (s, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 157.25 (d, J = 247.5 Hz), 140.50, 129.50 (d, J = 7.5 Hz), 128.30, 121.41 (d, J = 18.75 Hz), 115.11-115.73 (m), 67.66-68.02 (m), 20.00-20.98 (m).

3-(1-(2,6-Dichloro-3-fluoro[5-D]phenyl) [1,2,2,2-D$_4$]ethoxy)-2-nitropyridine (10)

To a stirred solution of triphenylphosphine (11.45 g, 43.66 mmol) and DEAD (7.61 g, 43.70 mmol) in THF (30 mL) at 0 °C was added a solution of 1-(2,6-dichloro-3-fluoro[5-D]phenyl) [1,2,2,2-D_4]ethan-1-ol (**8**, 6.18 g, 28.87 mmol) and 3-hydroxynitropyridine (**9**, 4.08 g, 29.12 mmol) in THF (30 mL). The resulting bright orange solution was stirred under a nitrogen atmosphere at ambient temperature for 0.5 hour at which point all starting materials had been consumed. The solvent was removed, and the crude residue was purified by flash column chromatography (PE:EA = 2:1) to afford **10** (4.15 g, 42.8% yield) as an off-white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.03 (d, J = 5.0 Hz, 1H), 7.39 (dd, J = 8.5, 4.5 Hz, 1H), 7.31 (dd, J = 9.5, 5.0 Hz, 1H), 7.23 (d, J = 8.5 Hz, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 157.51 (d, J = 250.0 Hz), 149.56, 145.12, 139.53, 135.49, 130.09, 128.88 (d, J = 2.5 Hz), 128.18, 123.88, 121.91 (d, J = 18.8 Hz), 117.15 (d, J = 23.8 Hz), 74.50, 17.94-18.34 (m).

3-(1-(2,6-Dichloro-3-fluorophenyl-5-[D]) [1,2,2,2-D$_4$]ethoxy-pyridin-2-amine (11)

To a stirred mixture of AcOH (25 mL) and **10** (4.10 g, 12.20 mmol) was added zinc powder (15.7 g, 240.06 mmol). The mixture was heated to 60 °C and allowed to stir for 30 minutes. The mixture was cooled to room temperature and carefully neutralized by the addition of a saturated Na_2CO_3 aqueous solution. The mixture was extracted with EtOAc

(100 mL x 2). The combined organic layers were washed with brine, dried with $MgSO_4$ and evaporated to dryness. The crude residue was purified by flash column chromatography (PE:EA = 1:1) to afford **11** (3.15 g, 84.5% yield) as an off-white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.60 (d, J = 5.0 Hz, 1H), 7.28 (d, J = 4.5 Hz, 1H), 6.71 (dd, J = 8.0, 1.0 Hz, 1H), 6.47 (dd, J = 7.5, 5.0 Hz, 1H), 4.89 (br s, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 157.42 (d, J = 248.8 Hz), 150.56, 139.81, 138.83, 137.06, 129.83, 128.70 (d, J = 41.3 Hz), 121.97 (d, J = 18.8 Hz), 116.97, 116.36 (t, J = 28.8 Hz), 113.38, 71.86-72.04 (t, J = 22.5 Hz), 18.17 (q, J = 20.0 Hz).

5-Bromo-3-(1-(2,6-dichloro-3-fluoro[5-D]phenyl) [1,2,2,2-D_4] ethoxy) pyridin-2-amine (12)

N-Bromosuccinimide (NBS, 1.78 g, 0.01 mol) was added portionwise to a stirred solution of **11** (3.06 g, 0.01 mol) in acetonitrile (50 mL) at 0 °C. After the resulting mixture was stirred at 0 °C for 10 minutes, it was concentrated to dryness under vacuum. The residue was diluted with EtOAc (100 mL). The organic layer was washed with brine, dried with $MgSO_4$ and evaporated to dryness. The crude residue was purified by flash column chromatography (PE:EA = 1:1) to afford **12** (3.00 g, 77.9% yield) as an off-white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.67 (d, J = 2.5 Hz, 1H), 7.32 (d, J = 4.5 Hz, 1H), 6.85 (d, J = 1.5 Hz, 1H), 4.91 (br s, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 157.50 (d, J = 248.8 Hz), 149.31, 140.03, 139.33, 136.35, 129.93, 128.89 (d, J = 3.8 Hz), 121.98 (d, J = 20.0 Hz), 119.98, 116.76 (t, J = 22.5 Hz) , 106.61, 72.49 (t, J = 22.5 Hz), 18.12 (dd, J = 32.5, 18.8 Hz). HRMS (ESI+, $[M+H]^+$) *m/z*: 383.9739 .

***tert*-Butyl 4-((methylsulfonyl)oxy) [2,2,6,6-D_4]piperidine-1-carboxylate (13)**

To a stirred solution of *tert*-butyl 4-[2,2,6,6-D_4]hydroxypiperidine-1-carboxylate (**5**, 6.10 g, 29.71 mmol) in DCM (60 mL) was slowly added Et_3N (4.21 mL, 30.29 mmol) first and then methane sulfonyl chloride (4.44 g, 38.76 mmol) in DCM (5 mL) at 0 °C. The mixture was stirred at room temperature for 2 hours and then quenched with water (25 mL). The aqueous layer was extracted with DCM (100 mL x 2). The combined organic layers were

washed with brine, dried with $MgSO_4$ and evaporated to dryness. The crude residue was purified by flash column chromatography (PE:EA = 1:2) to afford **13** (7.24 g, 86.0% yield) as an light yellow solid. HRMS (ESI+, $[M+H]^+$) *m/z*: 284.1456.

***tert*-Butyl 4-(4-iodo-1*H*-pyrazol-1-yl) [2,2,6,6-D_4]piperidine-1-carboxylate (15)**

To a stirred solution of *tert*-butyl 4-((methylsulfonyl)oxy) [2,2,6,6-D_4]piperidine-1-carboxylate (**13**, 2.00 g, 7.06 mmol) and 4-iodopyrazole (**14**, 1.34 g, 6.91 mmol) in NMP (15 mL) was added cesium carbonate (3.0 g, 9.21 mmol). The mixture was heated to 80 °C and allowed to stir for 4 hours. The reaction solution was diluted with EtOAc (200 mL) and washed with water (100 mL). The organic layer was separated and washed with brine, dried with $MgSO_4$ and evaporated to dryness. The crude residue was purified by flash column chromatography (PE:EA = 2:1) to afford **15** (1.86 g, 70.7% yield) as a colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.53 (s, 1H), 7.47 (s, 1H), 4.26-4.32 (m, 1H), 2.09-2.12 (m, 2H), 1.88 (t, J = 12.0 Hz, 2H), 1.49 (s, 9H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 154.54, 144.00, 131.26, 79.96, 59.76, 55.84, 32.09, 28.41 (*note: ^{13}C-NMR peaks for carbons connected with deuterium atoms were not showing up*). HRMS (ESI+, $[M+H]^+$) *m/z*: 382.0867.

***tert*-Butyl 4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazol-1-yl) [2,2,6,6-D_4]piperidine-1-carboxylate (16)**

Bis(pinacilato)diboron (1.78 g, 7.01 mmol) and potassium acetate (1.93 g, 19.67 mmol) were added sequentially to a solution of **15** (1.86 g, 4.88 mmol) in DMSO (20 mL). After the mixture was purged with nitrogen three times, dichlorobis(triphenylphosphino)palladium(II) (0.2 g, 0.28 mmol) was then added to the mixture. The resulting mixture was heated at 80 °C for 2 hours and then cooled to ambient temperature. The mixture was filtered through a bed of Celite and the solid residue in a funnel was washed with EtOAc (5 mL x2). The filtrate was washed with brine, dried with $MgSO_4$ and evaporated to dryness under vacuum. The crude residue was purified by flash column chromatography (PE:EA = 3:1) to afford **16** (1.17 g, 62.9% yield) as a yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.75 (s, 1H),

4.32-4.36 (m, 1H), 2.12-2.15 (m, 2H), 1.89 (t, *J* = 11.5 Hz, 2H), 1.48 (s, 9H), 1.33 (s, 12H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 154.58, 144.84, 133.58, 83.40, 79.88, 59.07, 32.15, 28.42, 24.86 (*note: ^{13}C-NMR multi-peaks for carbons connected with deuterium and boron atoms are buried in the spectrum background*). HRMS (ESI+, $[M+H]^+$) *m/z*: 382.2731.

***tert*-Butyl 4-(4-(6-amino-5-(1-(2,6-dichloro-3-fluoro[5-D]phenyl)ethoxy-[1,2,2,2-D$_4$])pyridin-3-yl)-1*H*-pyrazol-1-yl) [2,2,6,6-D$_4$]piperidine-1-carboxylate (17)**

A solution of *tert*-butyl 4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazol-1-yl) [2,2,6,6-D$_4$]piperidine-1-carboxylate (**16**, 1.60 g, 4.20 mmol) and 5-bromo-3-(1-(2,6-dichloro-3-fluorophenyl-5-D) [1,2,2,2-D$_4$] ethoxy) pyridin-2-amine (**12**, 1.53 g, 3.97 mmol) in toluene (12 mL) and water (12 mL) was purged with nitrogen gas a few times. Then, Pd(dppf)Cl_2 (180 mg, 0.25 mmol) , Cs_2CO_3 (3.90 g, 11.99 mmol) and tetraethylammonium bromide (70 mg, 0.33 mmol) were added to the above solution under nitrogen atmosphere. The resulting mixture was purged with nitrogen gas and stirred at 80 °C for 9 hours. The reaction solution was diluted with EtOAc (200 mL) and washed with water (100 mL). The organic layer was separated and washed with brine, dried with $MgSO_4$ and evaporated to dryness. The crude residue was purified by flash column chromatography (PE:EA = 1:2) to afford **17** (1.64 g, 73.9% yield) as an off-white solid. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.96 (s, 1H), 7.75 (d, *J* = 2.0 Hz, 1H), 7.54-7.57 (m, 2H), 6.91 (d, *J* = 2.0 Hz, 1H), 5.62 (s, 2H), 4.30-4.36 (s, 1H), 1.97-2.00 (m, 2H), 1.74 (t, *J* = 12.0 Hz, 2H), 1.42 (s, 9H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 154.32, 149.97, 139.28, 136.02, 135.13, 124.08, 119.69, 117.84, 115.01, 79.28. 58.51, 28.56 (*note: ^{13}C-NMR peaks for carbons connected with deuterium atoms are buried in the background*). HRMS (ESI+, $[M+H]^+$) *m/z*: 559.2354 .

***tert*-Butyl**

4-(4-(6-amino-5-(1-(2,6-dichloro-3-fluoro[5-D]phenyl)ethoxy[1,2,2,2-D_4])pyridin-3-yl)-1*H*-pyrazol-1-yl) [2,2,6,6-D_4]piperidine-1-carboxylate (18)

A solution of *tert*-Butyl 4-(4-(6-amino-5-(1-(2,6-dichloro-3-fluoro[5-D]phenyl)ethoxy-[1,2,2,2-D_4])pyridin-3-yl)-1*H*-pyrazol-1-yl) [2,2,6,6-D_4]piperidine-1-carboxylate (**17**, 700 mg, 1.25 mmol) in 5% HCl in MeOH (8 mL) was stirred at room temperature for 18 hours. The mixture was diluted with water (70 mL) and the aqueous layer was extracted with EtOAc (70 mL). The pH of aqueous layer was adjusted to about 11 by adding a NaOH solution, and then the aqueous layer was extracted with DCM (150 mL x 2). The combined organic layers were washed with brine, dried with $MgSO_4$ and evaporated to dryness. The crude residue was purified by flash column chromatography (DCM:MeOH = 20:1) to afford **18** (299 mg, 52.0% yield) ad an off-white solid. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.91 (s, 1H), 7.76 (d, J = 1.5 Hz, 1H), 7.57 (d, J = 5.0 Hz, 1H), 7.53 (s, 1H), 6.91 (d, J = 2.0 Hz, 1H), 5.62 (s, 2H), 4.14-4.18 (m, 1H), 2.99-3.07 (m, 2H), 1.92-1.95 (m, 2H), 1.74 (t, J = 12.0 Hz, 2H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 156.30, 149.91, 139.29, 137.29, 135.97, 134.94, 130.98, 129.20, 123.75, 121.53 (d, J = 18.8 Hz), 119.50, 117.95, 115.00, 59.40, 33.64 (*note: ^{13}C-NMR peaks for carbons connected with deuterium atoms are buried in the background*). HRMS (ESI+, $[M+H]^+$) *m/z*: 459.1815.

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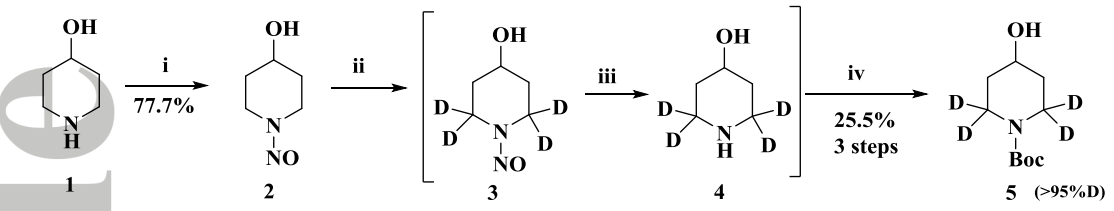
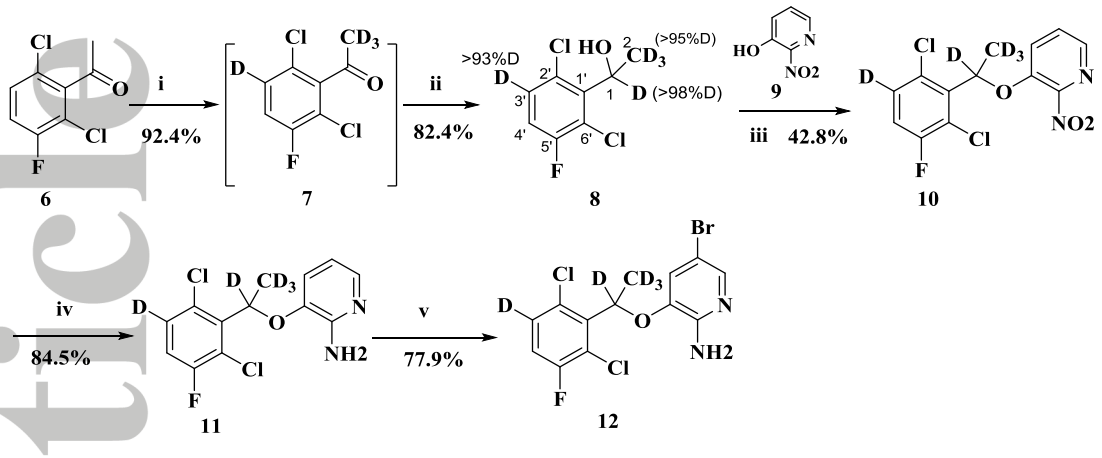
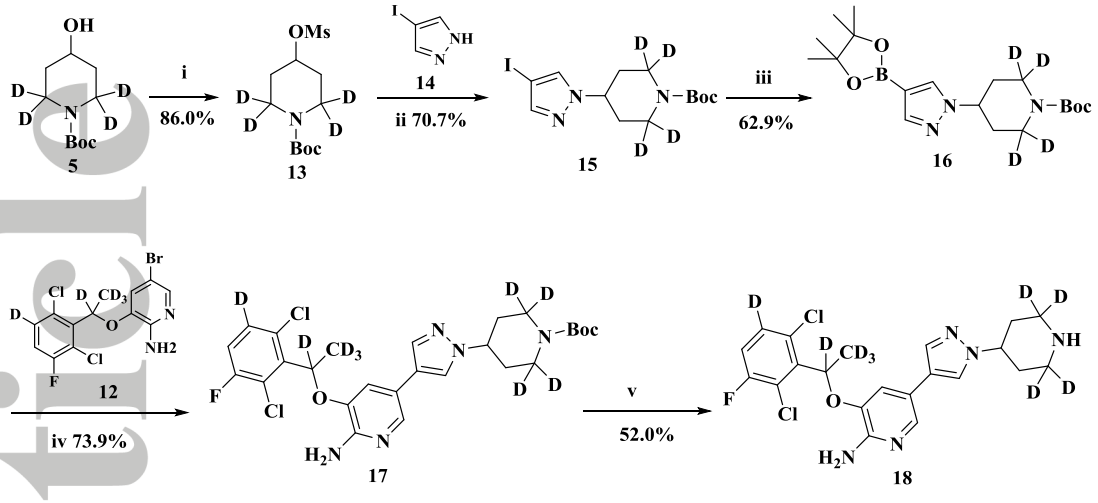


Scheme 1. Synthesis of *tert*-butyl 4-[2,2,6,6-D_4]hydroxypiperidine-1-carboxylate 5. reagents and conditions: (i) $NaNO_2$, HCl, -5 °C to ambient temperature, overnight; (ii) NaOMe, D_2O, 100 °C, 24 h; (iii) Al-Ni, 80 °C, 80 min; (iv) $(Boc)_2O$, RT, 4 h.



Scheme 2. Synthesis of the deuterated intermediate 12. reagents and conditions: (i) NaOMe, D_2O, 1,4-dioxane, 100 °C, 6 hours; (ii) $NaBD_4$, THF, CH_3OD, 60 °C, 1 hour; (iii) Ph_3P, DEAD, THF, 0 °C, 0.5 h; (iv) Zn, AcOH, 60 °C, 30 minutes; (v) NBS, ACN, 0 °C, 10 minutes.



Scheme 3. Synthesis of R/S-[D_9]crizotinib 18. reagents and conditions: (i) MsCl, Et_3N, DCM, RT, 2 hours; (ii) Cs_2CO_3, NMP, 80 °C, 4 hours; (iii) Bis(pinacilato)diboron, $Pd(Ph_3P)_2Cl_2$, KOAc, DMSO, 80 °C, 2 hours; (iv) $Pd(dppf)Cl_2$, Cs_2CO_3, Tetraethylammonium bromide, Toluene/H_2O, 80 °C, 9 h; (v) 5% HCl in MeOH, RT, 18 hours.

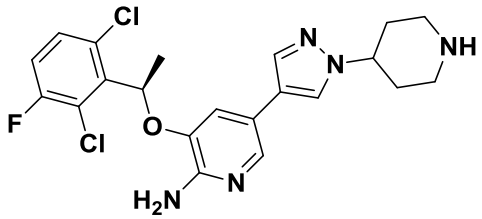


Figure 1 Structure of crizotinib

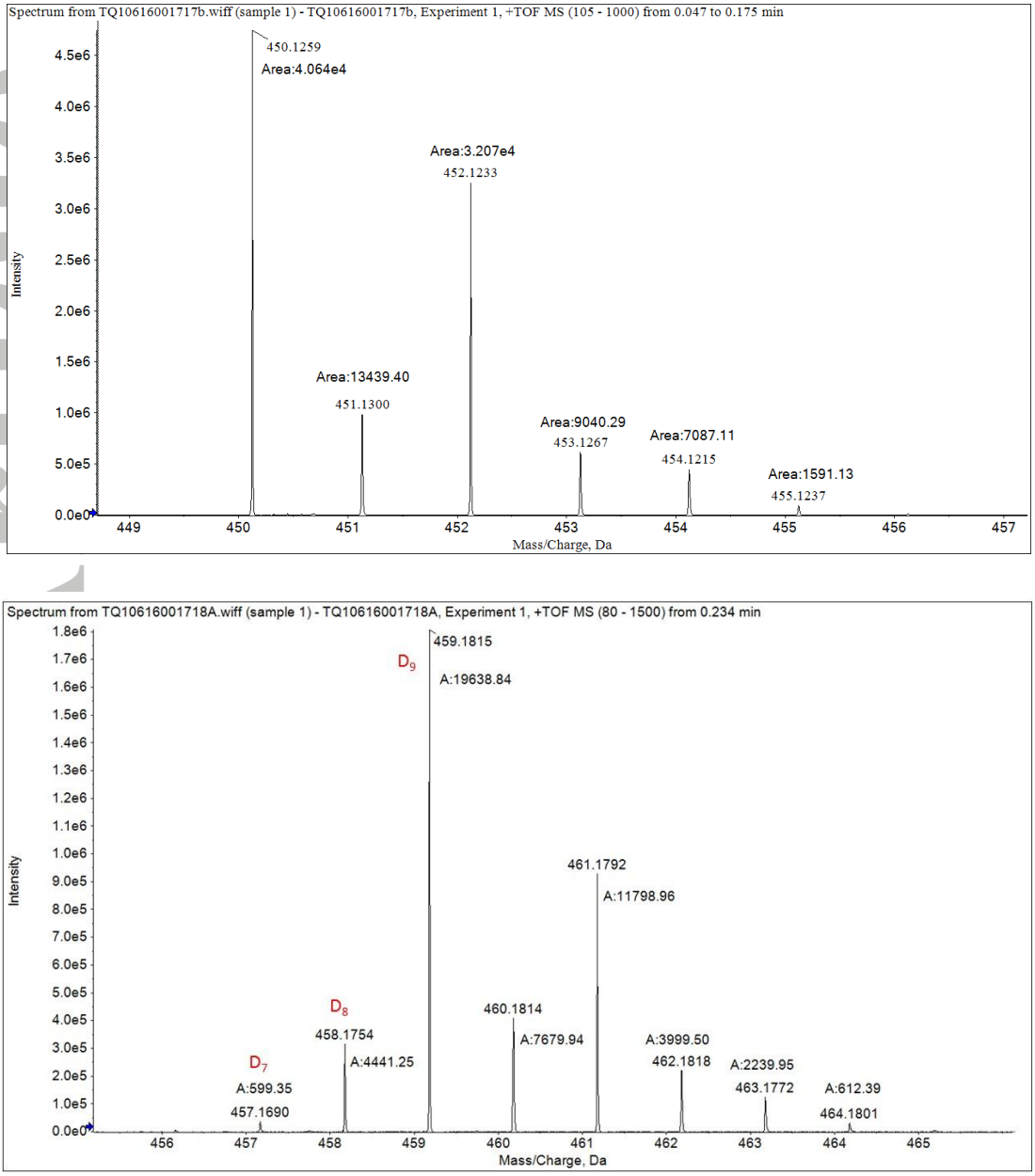


Figure 2 HRMS of crizotinib and [D_9]crizotinib