An efficient and facile synthesis of deuterium-labeled anticancer agent bendamustine hydrochloride

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Abstract: Bendamustine hydrochloride is an alkylating agent which was developed for the treatment of various human cancers. The stable isotope-labeled bendamustine was required to support clinic studies. An effective and operationally simple method for the synthesis of [D_6] bendamustine hydrochloride was developed using DCl as a catalyst and D_2O as a deuterium source. Under the present condition, regioselectively deuterated bendamustine hydrochloride with high deuterium incorporation is achieved.

Keywords: bendamustine; deuterium-labeling; internal standard; H-D exchange

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Introduction

Bendamustine hydrochloride (Fig.1) is a water-soluble, bifunctional alkylating agent with a benzimidazole ring that induces more long-lasting DNA double-strand breaks than other alkylating drugs.[1] It is reported that bendamustine had clinical activity against various human cancers including non–Hodgkin's lymphoma, multiple myeloma, chronic lymphocytic leukemia, breast cancer and small-cell lung cancer.[2] In addition, it is indicated that bendamustine is non–cross-resistant with alkylating agents and other drugs in vitro and in the clinic.[3, 4]

Compounds labeled with stable isotopes are most commonly used as internal standards for the quantification of drugs in biological samples.[5] Stable isotope labeled compounds also played an important role in the assessment of drug pharmacology to determine the pharmacokinetic profile or mode of action of a drug substance.[6] High quality information about the identity and quantification of drug-related compounds in biological samples can be provided by a well-designed labeled compound, which is extremely valuable at key decision points in drug development.

To our knowledge, there were a few procedures described in literature for the preparation of deuterium-labeled bendamustine.[7] For bendamustine, the abundance of the M+5 (362 amu) isotopologues comes to 1.9%, 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 bendamustine in MS.

Considering the structure of bendamustine, [D_6]bendamustine hydrochloride **2** and [D_6]Bendamustine hydrochloride **3** were first designed as our target compounds. However, we failed to synthesize [D_6]bendamustine hydrochloride **2** due to the decomposition of the intermediate during the H-D exchange reaction. At the same time, the route to prepare [D_6]bendamustine hydrochloride **3** was time-consuming and uncertain (11 steps starting from CD_3NH_2 and dihydro-2*H*-pyran-2,6(3*H*)-dione). Fortunately, an effective and facile method was found to synthesize [D_6]bendamustine hydrochloride **4** when we explored ways to synthesize [D_6]bendamustine hydrochloride **2**.

Experimental

General

Unlabeled intermediate (**9**) and authentic reference standards were supplied by Process Chemistry Institute of Chia Tai Tianqing Pharmaceutical Group Co. All other reagents and solvents were commercially available and used without further purification. NMR spectra were recorded on a Bruker Avance 500 spectrometer. Chemical shifts (δ) in ppm are quoted relative to $CDCl_3$, D_2O or DMSO-d_6. High-resolution mass spectra were recorded on AB SCIEX Triple TOF 4600. TLC analysis and

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coloration was performed using UV light (254 nm) or phosphomolybdic acid. HPLC was obtained on an Agilent HPLC using ZORBAX Eclipse XDB-C18, 150mm× 4.6mm, 5 μm column, and solvent which contained water, acetonitrile and trifluoroacetic acid. Microwave reactions were carried using a Discover SP-Microwave Synthesizer at max power of 250 W.

Ethyl 4-(5-amino-1-methyl-1*H*-benzo[*d*]imidazol-2-yl) butanoate (10)

A mixture of 4-(1-methyl-5-nitro-1*H*-benzoimidazol-2-yl)-butyric acid ethyl ester **9** (30.01 g, 103.02 mmol) and 10% palladium on charcoal (3.0 g) in methanol (400 mL) and ethyl acetate (200 mL) at room temperature was stirred under hydrogen atmosphere for 16 h. The resulting mixture was filtered and concentrated in vacuo to give compound **10** as a white solid (26.45 g, 98.3%) with 99.5% purity (HPLC). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.11 (d, J = 8.5 Hz, 1 H), 6.71 (d, J = 1.5 Hz, 1 H), 6.53 (m, 1 H), 4.66 (s, 2 H), 4.06 (q, J = 7 Hz, 2 H), 3.61 (s, 3 H), 2.79 (t, J = 7.5 Hz, 2 H), 2.44 (t, J = 7.5 Hz, 2 H), 1.98 (quintet, J = 7.5 Hz, 2 H), 1.18 (t, J = 7 Hz, 3 H); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 173.14, 153.86, 144.09, 143.84, 128.95, 111.39, 109.80, 102.64, 60.25, 33.35, 29.69, 26.12, 22.76, 14.59; HRMS ESI $(M+H)^+$ *m/z*: 262.1544.

4-(5-amino-1-methyl-1*H*-benzo[*d*]imidazol-2-yl-4,6-d_2)butanoic-2,2,4,4-d_4 acid hydrochloride (11)

To a solution of compound **10** (1.02 g, 3.90 mmol) in D_2O (15 mL) 35% DCl (1.64 g, 15.32 mmol) was added in a 35 mL microwave reaction vial. The vial was sealed and heated to 180°C in the microwave synthesis apparatus for 30 min. Then the mixture was evaporated to dryness *in vacuo.* To the residue was added fresh D_2O (15 mL) and another 35% DCl (1.01 g, 9.34 mmol). The resulting mixture was heated again to 180°C in the microwave synthesis apparatus for 30 min. The operation was repeated three times. The final residue was triturated with ethanol to afford compound **11** (1.01 g, 93.5%) as a gray solid (HCl salt) with 95% purity (HPLC). ^{1}HNMR (DMSO-d_6, 500 MHz): δ 8.04(s, 1H), 3.98 (s, 3 H), 2.04 (s, 2 H); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 174.13, 155.17, 131.66, 131.31, 130.37, 114.26, 31.78, 21.33; HRMS ESI $(M+H)^+$ *m/z*: 240.1482.

Ethyl 4-(5-amino-1-methyl-1*H*-benzo[*d*]imidazol-2-yl-4,6-d_2)butanoate-2,2,4,4-d_4 (12)

To a solution of compound **11** (7.51 g, 27.22 mmol) in ethanol-D (60 mL) thionyl chloride was added dropwise (4.84 g, 40.67 mmol) at 0°C. The mixture was kept at 0°C for 30 min and warmed to 50°C in

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an oil bath for 4 h. The reaction was monitored by TLC. After the completion of the reaction, the mixture was neutralized with saturated sodium bicarbonate solution and extracted with ethyl acetate (100 mL). The organic phases were dried over Na_2SO_4 and concentrated under vacuum to give compound **12** (5.92 g, 81.3%) as a gay solid with 97% purity (HPLC). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.13(s, 1 H), 4.85 (s, 2 H), 4.05 (q, *J* = 7 Hz, 2 H), 3.62 (s, 3 H), 1.95 (s, 2 H), 1.18 (t, *J* = 7 Hz, 3 H); ^{13}C NMR(DMSO-d_6, 125 MHz)：δ 173.15, 153.73, 144.06, 143.37, 128.80, 109.80, 60.24, 29.75, 22.46, 14.59; HRMS ESI $(M+H)^+$ *m/z*: 268.1929.

Ethyl 4-(5-(bis(2-hydroxyethyl)amino)-1-methyl-1*H*-benzo[*d*]imidazol-2-yl-4,6-d_2)butanoate-2,2,4,4-d_4 (13)

To a solution of compound **12** (5.92 g, 22.14 mmol) in acetic acid (26.61 g, 443.2 mmol) and D_2O (49.2 g, 2.46 mol) oxirane (7.72 g, 175.1 mmol) was added at 0°C. The mixture was kept at 0°C for 1 h and warmed to 25 °C in an oil bath for 18 h. After the completion of the reaction, the mixture was neutralized with saturated potassium carbonate solution and extracted with dichloromethane (3 × 50 mL). The organic phases were dried over Na_2SO_4 and concentrated under vacuum to afford the crude product as a yellow oil. To the crude product was added ethyl acetate and acetone (60 mL, v/v = 3:1), and after the resulting mixture was sonicated in an ultrasonic apparatus for 30 min the white solid crashed out. The product was filtered off and dried at 50°C under reduced pressure to give compound **13** as a white solid (6.16 g, 78.3%) with 97% purity (HPLC). ^{1}H NMR($CDCl_3$, 500MHz): δ 7.15(s, 1 H), 4.13 (q, *J* = 7 Hz, 2 H), 3.85 (t, *J* = 5 Hz, 4 H), 3.70 (s, 3 H), 3.57 (t, *J* = 5 Hz, 4 H), 2.13 (s, 2H), 1.26 (t, *J* = 7 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 125MHz): δ 173.25, 153.99, 144.71, 142.67, 128.95, 109.30, 60.45, 60.41, 56.23, 29.79, 22.37, 14.23; HRMS ESI $(M+H)^+$ *m/z*: 356.2494.

4-(5-(bis(2-chloroethyl)amino)-1-methyl-1*H*-benzo[*d*]imidazol-2-yl-4,6-d_2)butanoic-2,2,4,4-d_4 acid hydrochloride (4)

To a solution of compound **13** (6.01 g, 16.91mmol) in dichloromethane (80 mL) thionyl chloride (19.3 g, 162.14 mmol) was added dropwise at 0°C. The mixture was kept at 0°C for 1 h and warmed to 25°C in an oil bath for 9 h. The reaction was monitored by HPLC. After the completion of the reaction, the

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solvent was evaporated to afford the crude product **14** (7.90 g) as a black oil, which was used in the next step without further purification.

A solution of the aforementioned product **14** (7.90 g) in 35% DCl (53.10 g, 496 mmol) was refluxed in an oil bath for 2 h. The reaction was monitored by HPLC. After the completion of the reaction, the solvent was concentrated under vacuum to afford the crude product as a black oil. The crude product was added to D_2O (28 mL) and stirred at room temperature to give a gray solid (6.32 g), which was dissolved in 35% DCl (5 mL) and more D_2O (18 mL) at 60°C. Activated carbon (1.02 g) was added and stirring was continued for 30 min. The activated carbon was separated by filtration. The filtrate was evaporated to dryness. Another 15 mL of D_2O was added to the residue. The mixture was placed at 4°C for crystallization for 24 h. The precipitate was separated by filtration and washed with D_2O. The filter cake was dried at 50°C under reduced pressure to give the titled compound **4** (2.05 g, 30.2%) as a white solid with 99.1% purity (HPLC). ^{1}H NMR(DMSO-d_6, 500 MHz): δ 7.73 (s, 1 H), 3.91 (s, 1 H), 3.84 (t, J = 6 Hz, 4 H), 3.79 (t, J = 6 Hz, 4 H), 1.99 (s, 2 H). ^{13}C NMR(DMSO-d_6, 125 MHz): δ 174.23, 152.21, 146.00, 132.09, 125.18, 113.81, 52.82, 41.48, 31.42, 21.70. HRMS ESI $(M+H)^+$ m/z: 364.1486.

Results and discussion

In order to get [D_6]bendamustine hydrochloride, we first designed synthetic routes to prepare [D_6]bendamustine hydrochloride **2** (Scheme 1) and [D_6]bendamustine hydrochloride **3**. However, we failed to obtain the labeled compound **8** due to the decomposition of compound **7** under several reported H-D exchange conditions (DCl/D_2O, Pd/Pt/D_2O & [D]TFA/D_2O).[8, 9, 10] The nitro group of compound **7** was considered to be the trouble maker in this case. Thus, we attempted to use aniline compound **10** as a starting material of the H-D exchange reaction to introduce 2 to 3 deuteriums into the benzene ring. 4-(5-amino-1-methyl-1H-(4, 6-D_2)benzo[*d*]imidazol-2-yl) butanoic acid was expected as a desired labeled compound based on the reported method.[8] However, deuteriums were introduced not only into the expected positon of the benzene ring, but also into the benzyl position and alpha position of the carboxylic acid group. Thus, compound **11** with six deuteriums incorporated was obtained. With the

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labeled compound **11** in hand we were able to successfully synthesize [D_6]bendamustine hydrochloride **4** via the route depicted in Scheme 2.

Reagents and conditions: (i) CD_3NH_2.HCl, AcONa, DMF; (ii) Na_2S, $NaHCO_3$, H_2O, reflux; (iii) a) D_2O, 35% DCl, MW, 150-180 °C, 30 min or b) Pd/Pt/D_2O MW,150-180 °C, 30 min or c) [D]TFA/D_2O, MW, 150-180 °C, 30 min.

Briefly, unlabeled aniline compound **10** was prepared by reduction of nitro compound **9** with 10% Pd/C catalyst in 98% yield. This compound was then treated with D_2O and 35%DCl under microwave heating condition to give deuterium-labeled compound **11** in 94% yield after 3 exchange cycles. The acid **11** was converted to the ethyl ester **12** in 82% yield using $SOCl_2$ and ethanol. Double *N*-alkylation of **12** with ethylene oxide offered the compound **13** in 78% yield. Double chlorination of **13** with $SOCl_2$ gave the dichloride **14**, which was immediately hydrolyzed with a D_2O solution of DCl to give the desired compound **4 ([D_6]**bendamustine hydrochloride) in 32% yield. It should be noted that compound **14** was very unstable in aqueous media,[11] and the crude material should be hydrolyzed to form an HCl salt as soon as possible.

Reagents and conditions: (i) H_2, 10% Pd/C, MeOH/Ethylacetate, rt, 16 h, 98.3%; (ii) D_2O, 35% DCl, MW, 180 °C, 30 min, three cycles, 93.5%; (iii) $SOCl_2$, EtOD, 50 °C, 4 h, 81.3%; (iv) Ethylene oxide, AcOH, D_2O, 25 °C, 78.3%; (v) $SOCl_2$, 9 h; (vi) 35% DCl, D_2O, reflux, 30.2%.

The ^{1}H NMR spectroscopic analysis of the compound **11** subjected to the H-D exchange condition for one cycle revealed that protons ortho to aniline nitrogen and methylene protons αto the benzimidazole had undergone a complete H-D exchange. In addition, to some extent, also the methylene protons α to the carboxyl function had also been replaced by deuteriums (~50%, determined by ^{1}HNMR, Figure 1). After three cycles all the protons mentioned above had undergone an almost complete H-D exchange (deuterium enrichment >98%, Figure 2 and Figure 3) to give the compound **11**. The structure of the final target, [D_6]bendamustine hydrochloride **4,** was confirmed by ^{1}H & ^{13}C NMR and HRMS (Figure 4).

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Conclusion

In summary, we have developed an efficient method for the preparation of deuterium-labeled bendamustine hydrochloride **4**. The key labeled intermediate **11** with six deuteriums was prepared using direct H-D exchange reaction with a catalyst of DCl and a deuterium source of D_2O. This efficient labeling method allowed us to quickly access the qualified deuterium-labeled bendamustine hydrochloride **4** as an internal standard.

Acknowledgements

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Conflict of interest

The authors confirm that this article content has no conflicts of interest.

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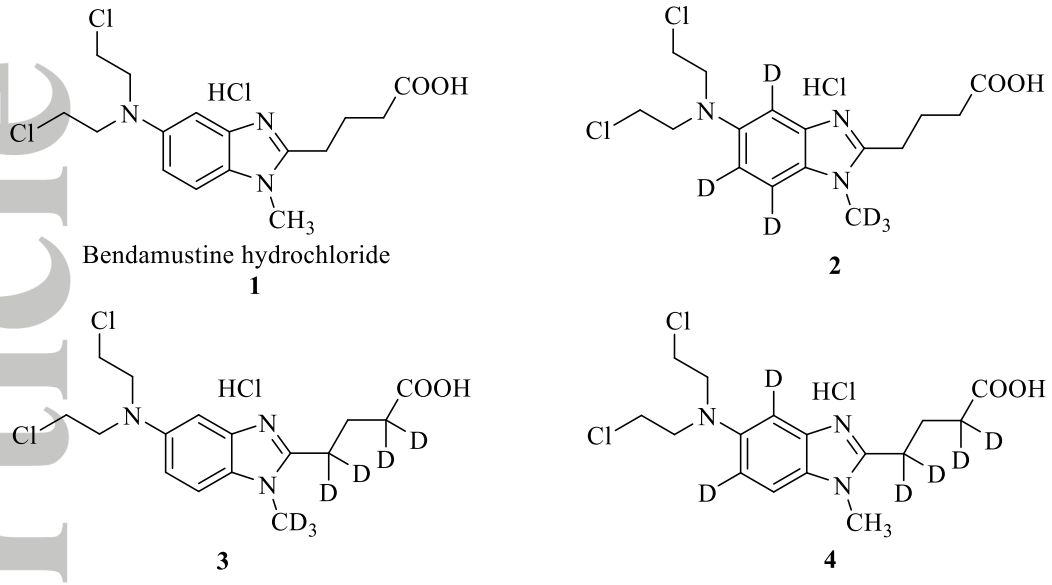

Figure 1. Bendamustine hydrochloride and its deuterium-labeled compounds **1-4**

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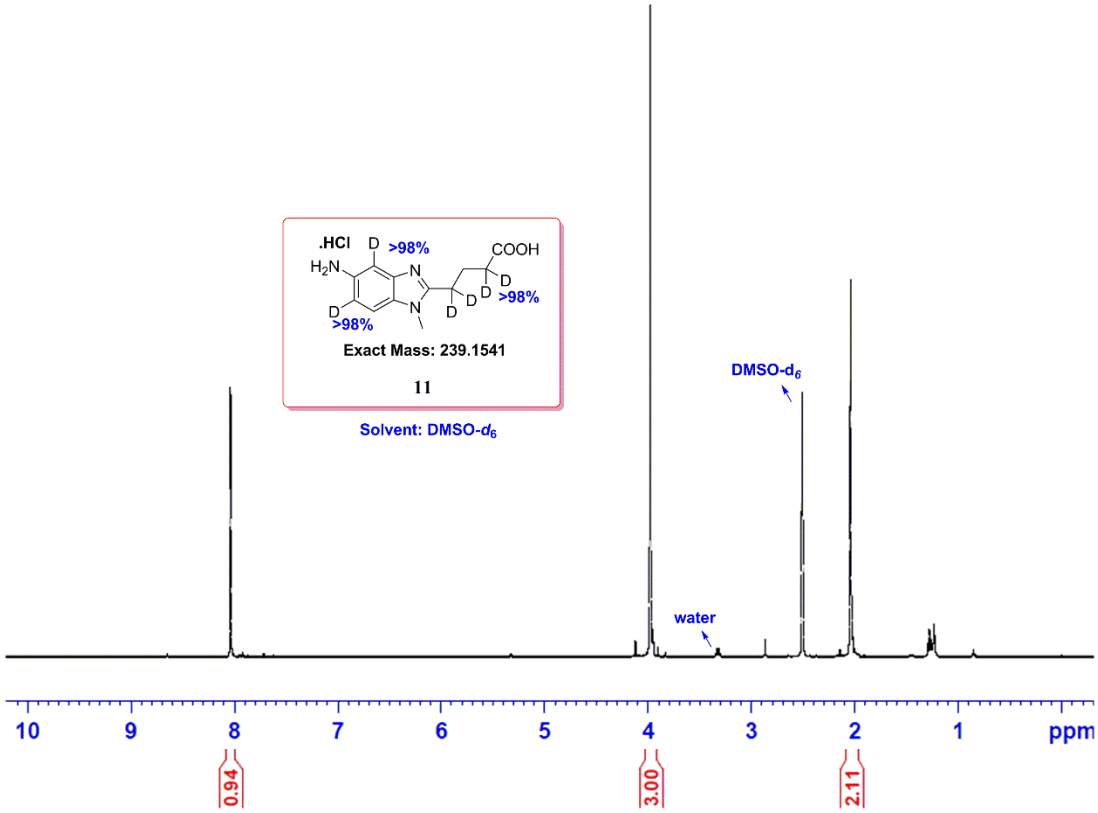

Figure 2. ^{1}H NMR-spectra of compound **11** after three H-D exchange cycles

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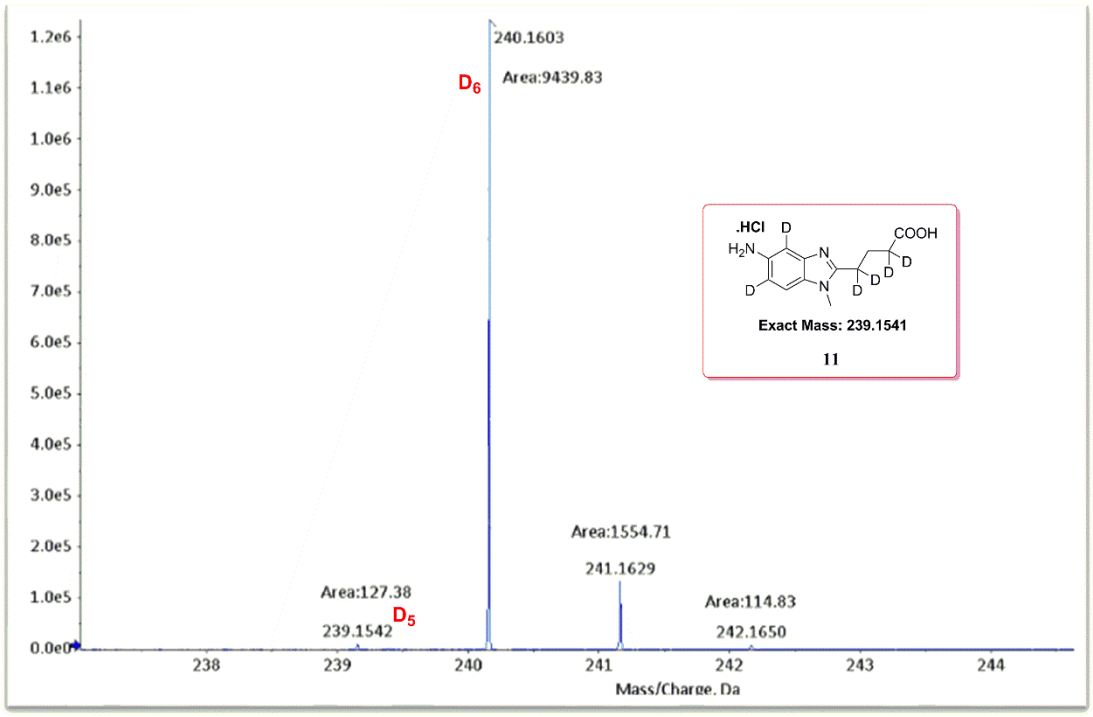

Figure 3. HRMS of compound **11** after three H-D exchange cycles

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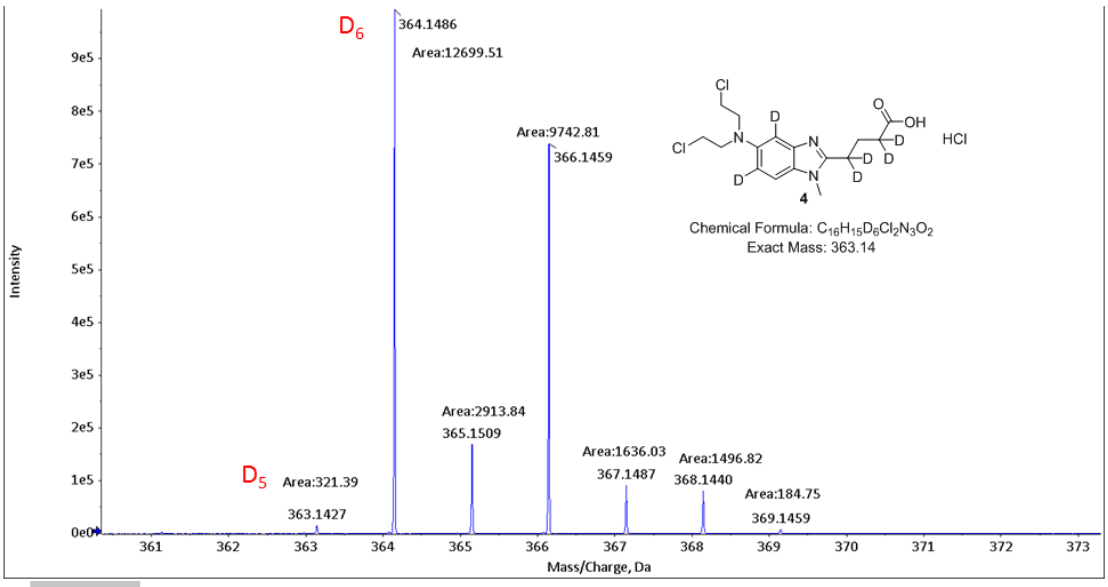

Figure 4. HRMS of compound **4**

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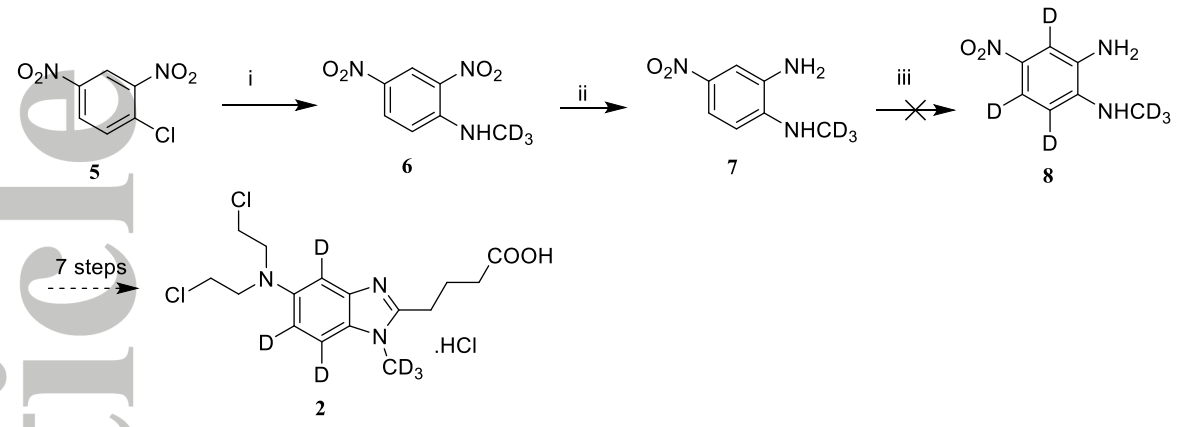

Scheme 1. Synthetic route of [D_6]bendamustine hydrochloride **2**.

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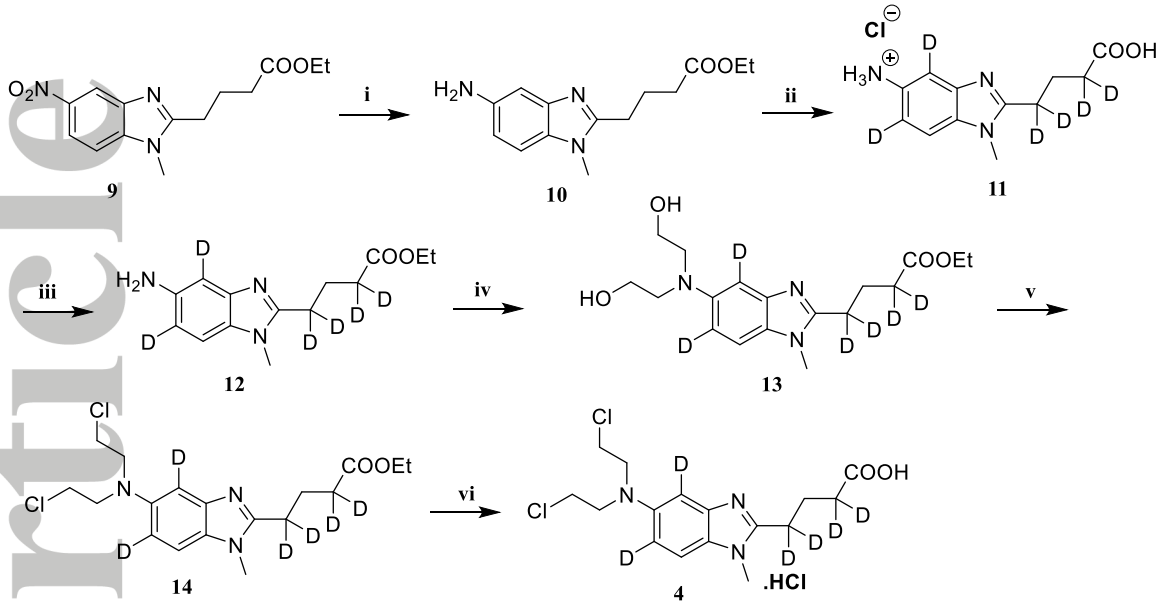

Scheme 2. Synthesis of [D_6]bendamustine hydrochloride **4**.

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Graphic Abstract

An efficient and facile synthesis of deuterium-labeled anticancer agent bendamustine hydrochloride

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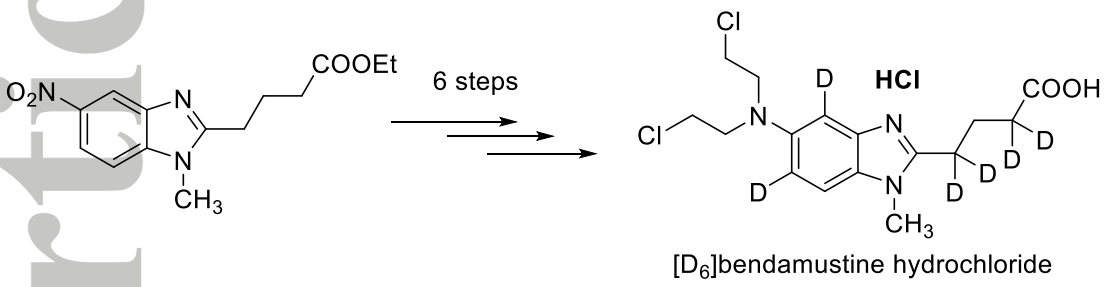

The stable isotope-labeled bendamustine was required to support clinic studies. An effective and operationally simple method for the synthesis of [D_6]bendamustine hydrochloride was developed using DCl as a catalyst and D_2O as a deuterium source. Under the present condition, regioselectively deuterated bendamustine hydrochloride with high deuterium incorporation is achieved

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