Synthesis of a potential bendamustine deschloro dimer impurity

Jie Yuan and Hai-Bin Zhu

Abstract
Bendamustine deschloro dimer was considered as a potential impurity in bendamustine hydrochloride resulting from the hydrolysis of bendamustine followed by intermolecular esterification. An efficient synthesis of bendamustine deschloro dimer was achieved from 4-(5-amino-1-methyl-1H-benzo[d]imidazol-2-yl)butanoate involving nine sequential steps including benzyl-protection/deprotection of the amine and carboxylic acid groups, saponification, ring-opening reaction of oxirane as well as Fischer/Steglish esterification and so on. The target bendamustine deschloro dimer was obtained using a high-performance liquid chromatography in a purity of 95.63%.

Keywords
bendamustine hydrochloride, bendamustine deschloro dimer, impurity reference, synthesis

Introduction
Bendamustine (BM) hydrochloride is a bifunctional alkylating agent used to treat patients with chronic lymphocytic leukaemia. It features an active nitrogen mustard moiety (mechlorethamine unit) together with a benzimidazole ring and a butanoic acid residue.1–4 Its mechlorethamine moiety is highly active in forming covalent bonds with DNA, causing DNA damage and leading to cell death.5,6

During the synthesis and storage of active pharmaceutical ingredients (APIs), impurities including organic/inorganic species as well as residual solvents may exist that have a great influence on the quality and safety of the pharmaceuticals.7 Owing to the presence of the intrinsically reactive nitrogen mustard moiety, BM is so moisture-sensitive that hydrolysis of mechlorethamine unit can easily occur to generate hydrolyzed species, namely monoehydroxy and dihydroxy derivatives (a and b),8 which can be further coupled to each other via an ester linkage, giving rise to BM dimer impurities, namely BM1 dimer and BM deschloro dimer (Scheme 1). According to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) regulations, it is mandatory to identify and control the level of impurities before final approval for commercialization of a pharmaceutical.9

In this context, we report the synthesis of BM deschloro dimer, starting from ethyl 4-(5-amino-1-methyl-1H-benzo[d]imidazol-2-yl)butanoate (compound 1), which was efficiently obtained in nine reaction steps (Scheme 2).

Results and discussion
4-(5-Amino-1-methyl-1H-benzo[d]imidazol-2-yl)butanoate (compound 1) was first converted into mono-benzyl (4)- and bis-benzyl (6)-protected intermediate in different methods. Compound 4 was prepared via the reductive amination reaction involving a one-pot reaction of benzaldehyde in the presence of sodium cyanoborohydride (NaBH₃CN),10 which was subsequently reacted with oxirane to give compound 2. Compound 2 underwent saponification to afford compound 3, which had its carboxylic acid group protected by the Bn protecting group through a Fischer esterification reaction (benzyl alcohol/H₂SO₄), leading to the key intermediate of 4 with a hydroxyl reactive site. By contrast, the bis-benzyl-protected intermediate (5) was obtained through a direct N-benzylation reaction using excess benzyl bromide under basic conditions ((iPr)₂NEt/CH₃CN).11 Upon saponification, compound 5 was transformed into another key intermediate (6) bearing the naked carboxylic acid group. The two key intermediates (4 and 6) were then coupled to each other via an ester linkage, that is, the carboxylic acid group of compound 6 reacted with the hydroxyl group of compound 4 under Steglich esterification condition (DCC/
DMAP: DCC=dicyclohexyl-carbodiimide; DMAP=N,N-
dimethylaminopyridine) to produce the dimer of compound 7. Finally, the four Bn protecting groups in compound 7 were completely and cleanly removed in a one-pot reaction through a catalytic hydrogenolysis (Pd/C, H2) under a mild condition. Finally, the amino groups reacted with oxirane via the ring-opening polymerization (ROP) reaction and generating the target BM deschloro dimer.

**Conclusion**

In brief, a convenient and efficient synthetic route was developed towards the potential BM deschloro dimer impurity of BM hydrochloride. The key strategy of the current synthesis lies in employing benzyl groups to mask the reactive amine and carboxylic acid groups in the two key intermediates. These can be cleanly removed in a one-pot manner by mild hydrogenolysis after coupling of two intermediates. Our current work offers a facile method to gain high-quality BM deschloro dimer impurity reference for quality control of BM hydrochloride although BM deschloro dimer was not found as an impurity in bendamustine hydrochloride.

**Experiment**

All chemicals and solvents were commercially available and used without further purification. Flash column chromatography and thin-layer chromatography (TLC) inspections were performed on Aladdin silica gel (300–400 mesh) and silica gel plates (GF254), respectively. 1H NMR spectra were recorded on Bruker AV-300 and DRX-500 spectrometers. Electrospray ionization mass spectra (ESI-MS) were obtained on Agilent 6540 UHD Accurate-Mass Q-TOF ESI-MS and Finigan MAT SSQ710ESI spectrometers. High-performance liquid chromatography analysis was conducted on a Shimadzu LC-10ATvp/10ADvp.

**Preparation of ethyl 4-(5-(benzyl)(2-hydroxyethyl)amino)-1-methyl-1H-benzo[d]imidazol-2-yl)butanoate (2)**

A mixture of ethyl 4-(5-amino-1-methyl-1H-benzo[d]imidazol-2-yl)butanoate (compound 1) (5.0 g, 19.1 mmol), benzaldehyde (2.4 g, 22.6 mmol) and 20 drops of acetic acid in 150 mL of methanol was stirred at room temperature for 0.5 h. Upon addition of NaBH4CN (1.5 g, 23.9 mmol), the above reaction further continued for 1.5 h. The reaction mixture was poured into 100 mL of water, and extracted with dichloromethane. The combined organic extracts were washed with saturated brine and dried over anhydrous Na2SO4. After evaporation of solvent under reduced pressure, the resultant oily liquid was dissolved into a mixture of water (60 mL) and acetic acid (60 mL), and cooled to 0°C. 11.5 mL of oxirane was added dropwise into the solution, and the resultant solution was gradually warmed to room temperature and stirred overnight. The reaction pH was adjusted to 8–9 and extracted using dichloromethane. The organic extracts were combined, washed with saturated brine and dried over anhydrous Na2SO4. Finally, the four Bn protecting groups in compound 7 were completely and cleanly removed in a one-pot reaction through catalytic hydrogenolysis (Pd/C, H2) under a mild condition. Finally, the amino groups reacted with oxirane via the ring-opening polymerization (ROP) reaction and generating the target BM deschloro dimer.

**Preparation of 4-(5-(benzyl)(2-hydroxyethyl)amino)-1-methyl-1H-benzo[d]imidazol-2-yl)butanoic acid (3)**

Compound 2 (7.0 g, 17.7 mmol) was added into 60 mL of NaOH solution (10 wt%), and stirred at room temperature overnight. The resultant clear solution was concentrated to 15 mL under the reduced pressure followed by adjusting pH to 5–6 with acetic acid. The precipitates were filtered by suction and dried at 50°C under vacuum. Compound 3 was obtained as white solid (4.3 g, 66%); 1H NMR (300 MHz, CDCl3) δ 8.02 (m, 6H), 7.28 (m, 6H), 7.07 (s, 1H), 6.84 (m, 1H), 5.63 (s, 2H), 4.12 (m, 2H), 3.74 (t, 2H, J = 5.58 Hz), 3.24 (t, 2H, J = 7.26 Hz), 1.94 (m, 2H); MS (ESI) m/z calcld for C23H21N3O4+: 396.2; found 396.2.
of 25 drops of concentrated sulfuric acid (98 wt%), the reaction was heated to 80°C and stirred for 2 h. After cooled to room temperature, the reaction was neutralized with NaOH solution (10 wt%) and extracted with ethyl acetate. The combined extracts were washed with saturated brine and dried over anhydrous Na2SO4. After solvent evaporation under reduced pressure, compound 4 was isolated as white solid (1.5 g, 48.7%) by flash chromatography (CH2Cl2/CH3OH = 50/1, v/v); 1H NMR (500 MHz, CDCl3) δ 7.37 (m, 5H), 7.28 (m, 2H), 7.23 (m, 4H), 7.12 (m, 1H), 6.87 (m, 1H), 5.31 (s, 1H), 5.11 (s, 2H), 4.59 (s, 2H), 4.08 (brs, 2H), 3.82 (t, 2H, J = 5.7 Hz), 3.65 (s, 3H), 3.59 (m, 2H, J = 5.7 Hz), 2.96 (t, 2H, J = 7.65 Hz), 2.55 (t, 2H, J = 6.75 Hz), 2.17 (m, 2H); MS (ESI) m/z calcd for C28H32N3O3 [M + H]+: 458.2; found 458.2.

Preparation of ethyl 4-(5-(dibenzylamino)-1-methyl-1H-benzo[d]imidazol-2-yl)butanoate (5)
A mixture of compound 1 (2.0 g, 7.7 mmol), benzyl bromide (4.6 g, 26.9 mmol), N,N-diisopropylethylamine (2.6 g, 20.1 mmol) in 100 mL of acetonitrile was stirred at room temperature for 24 h. The solvent was evaporated to dryness under vacuum, and the residue was subjected to flash chromatography (CHCl3/CH3OH = 50/1, v/v) to give compound 5 (2.2 g, 4.5 mmol) with 82.9% yield; 1H NMR (300 MHz, CDCl3) δ 7.24 (m, 10H), 7.07 (m, 1H), 7.06 (s, 1H), 6.81 (m, 1H), 4.65 (s, 4H), 4.11 (m, 3H), 3.65 (s, 3H), 2.87 (t, 2H, J = 7.56 Hz), 2.46 (t, 2H, J = 6.96 Hz), 2.16 (m, 2H), 1.23 (t, 3H, J = 7.11 Hz); MS (ESI) m/z calcd for C28H32N3O2 [M + H]+: 442.2; found 442.2.

Preparation of 4-(5-(dibenzylamino)-1-methyl-1H-benzo[d]imidazol-2-yl)butanoic acid (6)
Compound 5 (2.2 g, 4.5 mmol) was dissolved into 30 mL of methanol, and the reaction was heated to 80°C for 2 h after addition of 30 mL of 10 wt% NaOH solution. After cooled to room temperature, the white solid was precipitated from the solution when the pH was adjusted to 5–6 with acetic acid. The white precipitates were collected by suction and dried under vacuum at 50°C to give compound 6 (1.8 g, 87.8%); 1H NMR (300 MHz, CDCl3) δ 7.26 (m, 10H), 7.07 (m, 1H), 6.82 (m, 1H), 4.63 (s, 4H), 3.61 (s, 3H), 2.96 (t, 2H, J = 6.91 Hz), 2.44 (t, 2H, J = 6.33 Hz); MS (ESI) m/z calcd for C26H28N3O2 [M + H]+: 414.2; found 414.2.

Preparation of benzyl 4-(5-(benzyl(2-((4-(5-(dibenzylamino)-1-methyl-1H-benzo[d]imidazol-2-yl)butanoyl)oxy)ethyl)amino)-1-methyl-1H-benzo[d]imidazol-2-yl)butanoate (7)
A mixture of compound 4 (1.5 g, 3.3 mmol), compound 6 (2.0 g, 4.8 mmol) and 4-dimethylaminopyridine (DMAP)
(4.0 g, 32.7 mmol) was stirred in 150 mL of dichloromethane at room temperature for 0.5 h followed by addition of dicyclopentyl-carbodiimide (DCC) (1.4 g, 6.8 mmol). The resultant clear solution was further stirred overnight. After removal of the white precipitate by filtration, the filtrate was evaporated to dryness under reduced pressure, and the residue was purified by flash chromatography to afford compound 7 (1.8 g, 64.5%); \( \text{H NMR (300 MHz, CDCl}_3 \) \( \delta 7.24–7.35 \ (m, \ 20H), \ 7.05–7.10 \ (m, \ 4H), \ 6.82 \ (d, \ J = 8.8 \text{ Hz}), \ 5.11 \ (s, \ 2H), \ 4.66 \ (s, \ 4H), \ 4.58 \ (s, \ 2H), \ 4.30 \ (s, \ 2H), \ 3.69 \ (s, \ 2H), \ 3.55 \ (d, \ 6H, \ J = 17.7 \text{ Hz}), \ 2.82 \ (dt, \ 4H, \ J = 27.6, \ 7.35 \text{ Hz}), \ 2.53 \ (t, \ 2H, \ J = 6.5 \text{ Hz}), \ 2.41 \ (t, \ 2H, \ J = 6.5 \text{ Hz}), \ 2.08–2.18 \ (m, \ 4H); \ MS (ESI) m/z \) \( \text{calcd for C}_{54}\text{H}_{57}\text{N}_6\text{O}_4^+ \ [M + H]^+ \): 853.4; found 853.4.

Preparation of BM deschloro dimer

Compound 7 (1.8 g, 2.1 mmol) was dissolved into the mixture of water (50 mL) and acetic acid (50 mL). Upon addition of 5 wt% Pd/C (0.36 g), the reaction was subjected to hydrogenolysis (1 atm H$_2$, 25°C) overnight. The Pd/C catalyst was removed by filtration, and the filtrate was cooled to 5°C in an ice water bath. 10.0 mL of oxirane was then added into the solution, and the reaction was naturally warmed to room temperature and reacted overnight. BM deschloro dimer was afforded by preparative HPLC (1.0 g, 78% yield in two steps); \( \text{H NMR (300 MHz, D}_2\text{O) \ \delta 7.18 \ (s, \ 1H), 7.12 \ (s, \ 1H), 6.91 \ (t, \ 2H, \ J = 10.0 \text{ Hz}), 6.86 \ (d, \ 2H, \ J = 10.0 \text{ Hz}), 4.22- \ (d, \ 2H, \ J = 5.0 \text{ Hz}), 3.8–3.64 \ (m, \ 6H), 3.58 \ (t, \ 2H, \ J = 5.0 \text{ Hz}), 3.50–3.38 \ (6H, m), 3.31 \ (3H, s), 3.29 \ (3H, s), 2.53 \ (2H, t, J = 8.0 \text{ Hz}), 2.40 \ (2H, t, J = 6.5 \text{ Hz}), 2.36 \ (2H, t, J = 8.0 \text{ Hz}), 2.17 \ (2H, t, J = 7.5 \text{ Hz}), 1.90–1.72 \ (4H, m); \ MS (ESI) m/z \) \( \text{calcd for C}_{34}\text{H}_{47}\text{N}_6\text{O}_7^+ \ [M + H]^+ \): 625.3; found 625.3

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ORCID iD

Hai-Bin Zhu https://orcid.org/0000-0003-3903-9183

Supplemental material

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