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Synthesis and crystal structure of trifluoromethyl-containing bendamustine hydrochloride

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Abstract

A simple strategy to afford trifluoromethyl-containing bendamustine hydrochloride in 34% overall via nine simple steps including substitution, selective reduction, *N*-acylation, cyclization, esterification, nitro-reduction, *N*-dihydroxyethylation, chlorination, and acid-catalyzed hydrolysis from commercially available 2,4-dinitrochlorobenzene is described. The structures of the intermediates and target product are established on the basis of infrared, nuclear magnetic resonance, and high resolution mass spectrometer. Moreover, the structure of target product is also confirmed by X-ray crystal analysis, and further studies indicate that the existence of intermolecular O–H···Cl and N–H···Cl hydrogen bonds are effective in stabilization of the crystal structure.

Keywords

bendamustine hydrochloride, crystal structure, synthesis, trifluoromethyl group

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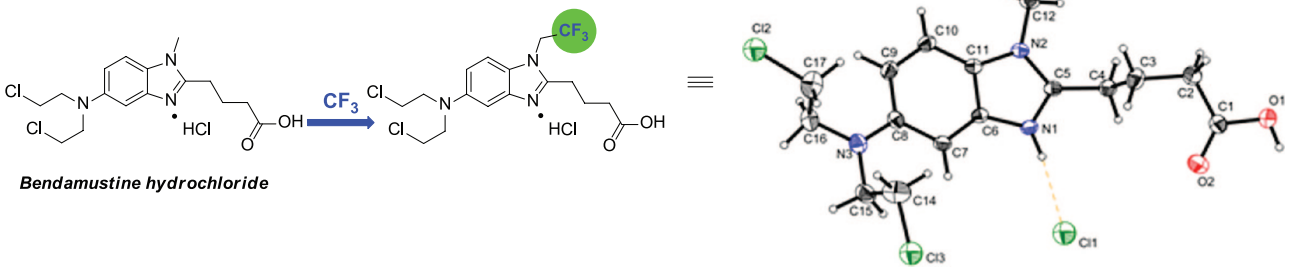

Bendamustine hydrochloride

Introduction

Bendamustine hydrochloride (Figure 1(a)) is a bifunctional molecule that combines the alkylating activity of a bis(2-chloroethyl)amine moiety and the antimetabolite activity of a benzimidazole group.[1–5] It causes DNA damage that is thought to lead to cell death, including inhibition of mitotic checkpoints and induction of mittic catastrophe.[6–8] In the 1960s, bendamustine hydrochloride was designed with the aim of creating a compound which possesses similar properties but less toxicity than nitrogen mustard agents.[9] This alkylating agent has been used against a number of malignancies since the 1970s in Germany.[10] It was approved by

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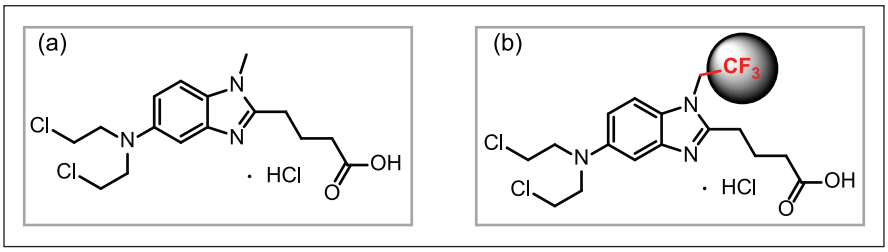


Figure 1. The chemical structures of (a) bendamustine hydrochloride and (b) trifluoromethylated bendamustine hydrochloride.

the US Food and Drug Administration (FDA) for the treatment of chronic lymphocytic leukemia in March 2008 and for rituximab-refractory indolent B-cell non-Hodgkin lymphoma (NHL) in October 2008.[9,11,12]

The small and highly electronegative fluorine atom can play a significant role in medicinal chemistry, biochemistry, and agrochemicals.[13,14] Around 20%–30% of medicines contain at least one fluorine atom.[15] Among the various fluorine-containing groups, the trifluoromethyl group (CF_3) has received significant attention as its introduction into pharmaceuticals has a major impact on the physical and chemical properties of the molecule such as lipophilicity, pharmacokinetics, and the binding properties of molecules.[16–20] Currently, the late-stage introduction of CF_3 in drug-like compounds is a well-known practice in medicinal chemistry and widely used in the treatment of diseases.[21,22] For example, lansoprazole and omeprazole are popular proton pump inhibitors (PPIs) used as anti-ulcer drugs.[23] The difference between these drugs is that lansoprazole contains a trifluoromethyl group, which can significantly improve drug stability and help to enhance its inhibitory effect on gastric acid secretion mechanism, thereby significantly improving the effect of clinical disease control.[24,25]

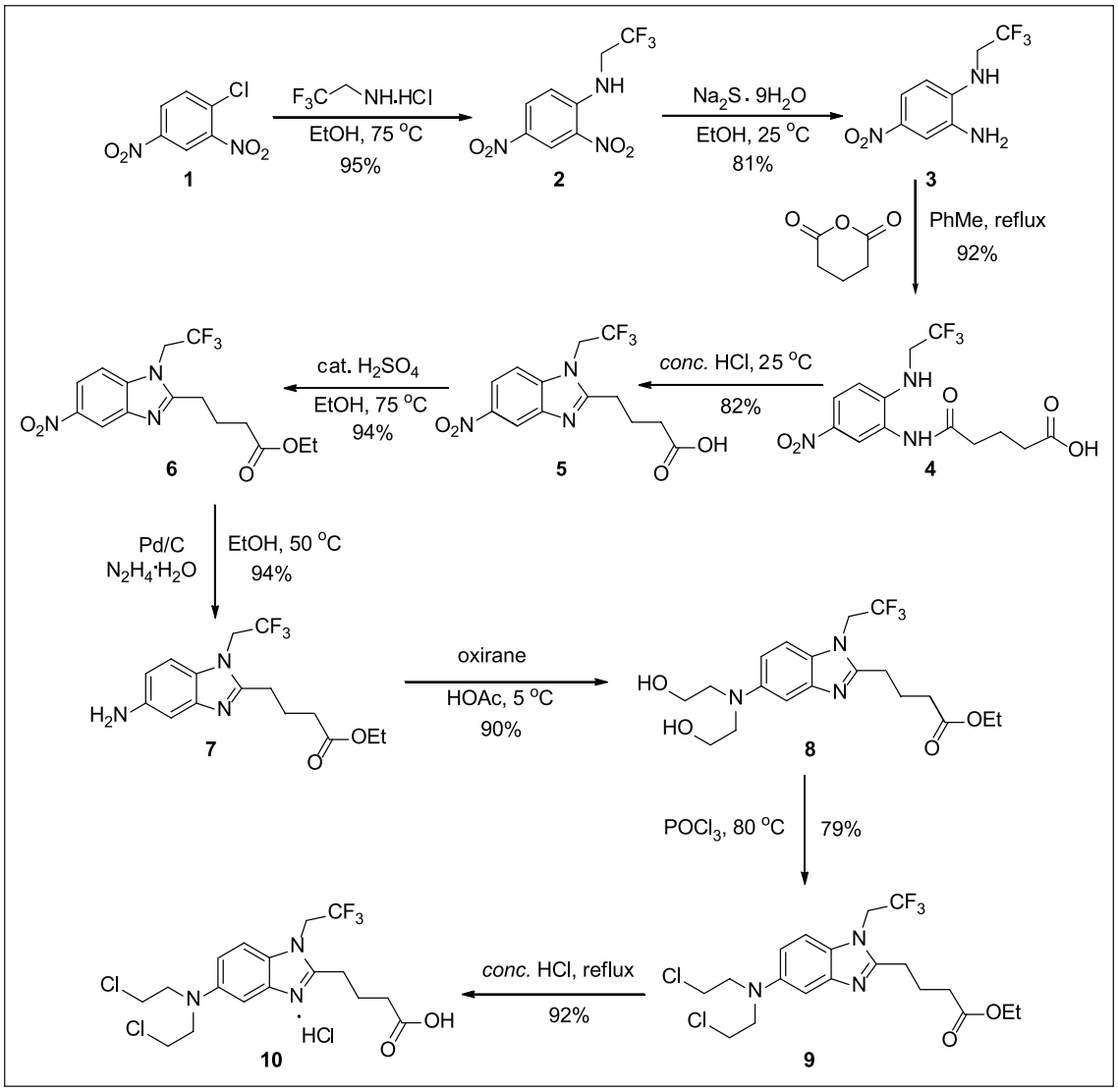
In this context, in view of the importance of bendamustine hydrochloride as an anti-tumor agent as well as the positive effect of CF_3 in organic molecules, it would be meaningful to introduce a CF_3 group on bendamustine hydrochloride (Figure 1(b)). In our continuing efforts for preparing important bioactive compounds,26 herein, using 2,4-dinitrochlorobenzene as a raw material, trifluoromethyl-containing bendamustine hydrochloride has been designed and synthesized by a series of organic synthesis steps including substitution, selective reduction, *N*-acylation, cyclization, esterification, nitro-reduction, *N*-dihydroxyethylation, chlorination, and acid-catalyzed hydrolysis (Scheme 1). This synthetic route involves simple operations and mild reaction conditions affording a 34% total yield.

Results and discussion

In order to introduce a trifluoromethyl group into bendamustine hydrochloride, our investigation began with the conversion of 2,4-dinitrochlorobenzene (**1**) into *N*-substituted dinitroaniline **2** via nucleophilic aromatic substitution S_NAr with 2,2,2-trifluoroethylamine. In 2016, Machara group reported a pathway to prepare the *N*-substituted dinitroaniline **2** and nitrophenyldiamine **3**; however, only 7% overall yield was obtained.27 To our delight, employing 2,2,2-trifluoroethylamine hydrochloride as a nucleophile, the desired product **2** was obtained in 95% yield. Moreover, the regioselective reduction of the *ortho*-nitro group could be realized in the presence of $Na_2S{\cdot}9H_2O$ and EtOH, which provided the corresponding nitrophenyldiamine **3** in 81% isolated yield. Next, to construct the imidazole ring and install the *n*-butyric acid group, glutaric anhydride was used to produce the desired amide product **4** in 92% isolated yield. To our delight, the imidazole ring was then smoothly formed through an intramolecular cyclization reaction using concentrated hydrochloric acid at room temperature, affording the desired product **5** in 82% isolated yield. In order to reduce the nitro to an amino group, the ester **6** was first obtained in 94% yield by the reaction of **5** in ethanol with catalytic sulfuric acid at 75 °C via a Fischer esterification process. Next, in the presence of Pd/C and hydrazine hydrate, the corresponding amino product **7** was formed. However, a hydrazide by-product appeared when the dropwise hydrazine hydrate addition was too fast. Therefore, hydrazine hydrate was added slowly at 50 °C, leading to a 94% yield of amino product **7**. Moreover, with the view of introducing dichloroethyl groups, first of all, reaction of excess oxirane with **7** in HOAc as the solvent resulted in the dihydroxyethyl compound **8** in 90% yield. Subsequently, the hydroxyl groups were exchanged for chlorine groups through the reaction of disubstituted product **8** with $POCl_3$ at 80 °C. The dichloroethyl product **9** was isolated in 79% yield. Finally, refluxing compound **9** in concentrated hydrochloric acid led to trifluoromethylated bendamustine hydrochloride **10** in 92% yield. It is also noteworthy that both compounds **9** and **10** may exhibit the potential toxicities acting as alkylation agents.

With the desired product **10** in hand, we successfully grew a single crystal from the mixture solvents of MeCN and EtOH, and the structure was confirmed by the X-ray crystal analysis (Figure 2(a), Cambridge Crystallographic Data Centre (CCDC) 1827727).

Compound 10 crystallizes in the $P2_1/c$ space group and its molecular structure contains one protonated trifluoromethyl-containing bendamustine cation and one free chlorine anion. Furthermore, the dihedral angle between the benzene ring and imidazole ring is 3.4(86)°, indicating that these rings are basically coplanar. Intermolecular O(1)–H(1O)···Cl(1) and N(1)–H(1N)···Cl(1) hydrogen bonds (Table 1) are formed between the free chlorine anions and trifluoromethyl-containing bendamustine and carboxyl carboxylic cations, and link the molecules forming a zig-zag



Scheme 1. The synthetic pathway to trifluoromethylated bendamustine hydrochloride **10**.

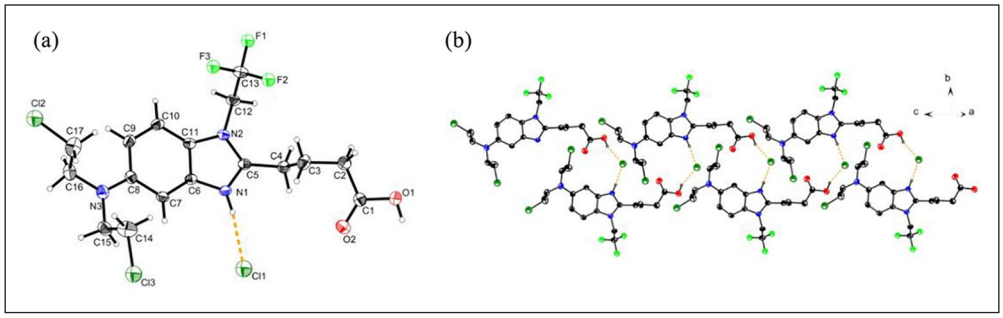


Figure 2. (a) The molecular structure of trifluoromethylated bendamustine hydrochloride **10** showing 30% probability of displacement ellipsoids and the atom-numbering scheme. (b) One-dimensional array running along the *c*-axis. Hydrogen bonds are shown as dashed lines.

chain along the *c*-axis, which is effective in stabilization of the crystal structure (Figure 2(b)).

Conclusion

In summary, using bendamustine hydrochloride as an original drug, trifluoromethyl-containing bendamustine hydrochloride was designed and synthesized. The target compound **10** was prepared from 2,4-dinitrochlorobenzene in nine steps in 34% overall yield. A study of the crystal structure of trifluoromethyl-containing bendamustine hydrochloride indicates that intermolecular hydrogen bonding interactions are effective in stabilization of the crystal structure, which may contribute evidence for studies of

Table 1. Distances and angles of possible weak interactions for compound **10**.

Atoms involved	Distance/Å		Angle/°
–	*D*···*A*	H···*A*	*D*–H···*A*
O(1)–H(1O)···Cl(1)[a]	3.020(2)	2.22(2)	156(4)
N(1)-H(1N)···Cl(1)	3.012(2)	2.185(14)	166(3)

Symmetry codes: [a]x,–y + 3/2, z − 1/2.

drug designs and mechanisms. Detailed biological activity studies of the title product are currently underway in our laboratory.

Experimental

General information

Unless noted otherwise, all the reagents were commercial available and were used without further purification. Column chromatography was performed on EMD Silica Gel 60 (200–300 Mesh) using a forced flow of 0.5–1.0 bar. Melting points were measured with an X4-A microscopic melting point apparatus. Nuclear magnetic resonance (NMR) spectra were recorded on a 300 MHz spectrometer with chemical shifts reported in ppm (in $CDCl_3$ or Dimethyl sulfoxide-d_6 (DMSO-d_6), with TMS as the internal standard). High resolution mass spectrometer (HRMS) were obtained on a 6540 Ultra High Definition (UHD) Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) liquid chromatography/mass spectrometry (LC/MS). Infrared (IR) spectra were recorded using a Thermo fisher Nicolet IS50 spectrometer. X-Ray single crystal diffraction data were recorded on a Bruker Smart APEX II CCD.

Preparation of 2,4-dinitro-N-(2,2,2-trifluoroethyl)aniline **(2)**

A mixture of 2,4-dinitrochlorobenzene (compound **1**) (20.26 g, 100 mmol), 2,2,2-trifluoroethylamine hydrochloride (16.26 g, 120 mmol), and triethylamine (22.26 g, 220 mmol) in ethanol was stirred in a high-pressure reactor at 75 °C for 48 h. After cooling, the solvent was evaporated under reduced pressure and the mixture was diluted with water and extracted with dichloromethane. The combined extracts were dried over Na_2SO_4 and concentrated under reduced pressure. Product **2** was obtained as a yellow solid (25.13 g, 95%); m.p. 114–115 °C (m.p. 119–121 °C);[27] IR (KBr) (ν_{max} cm^{-1}): 3366, 3110, 1620, 1592, 1523, 1367, 1343, 1321, 1294, 1253, 1165, 1128, 1057, 954, 928, 710, 674; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.17 (d, *J* = 2.6 Hz, 1H), 8.79 (s, 1H), 8.38 (dd, *J* = 9.5 Hz, 2.6 Hz, 1H), 7.10 (d, *J* = 9.5 Hz, 1H), 4.18–4.08 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 147.7, 137. 9, 132.0, 130.7, 123.9 (q, *J* = 278.5 Hz), 124.2, 114.0, 44.9 (q, *J* = 34.9 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ –71.2 (s, 3F); HRMS (electrospray ionization (ESI)) *m/z* calcd for $C_8H_5F_3N_3O_4^-$ $[M-H]^-$: 264.0232; found: 264.0238.

Preparation of 4-nitro-N^1-(2,2,2-trifluoroethyl)benzene-1,2-diamine **(3)**

A mixture of compound **2** (26.51 g, 100 mmol) and $Na_2S{\cdot}9H_2O$ (60.00 g, 250 mmol) in ethanol (200 mL) was stirred at 25 °C for 2 h. Aqueous $NaHCO_3$ solution (2.5 mol·L^{-1}, 100 mL) was added dropwise under stirring, and the mixture was stirred for 3 h. The crude product was precipitated from ice water and was washed with distilled water. Product **3** was obtained as a red-brown solid (18.94 g, 81%); m.p. 163–165 °C (m.p. 156–158 °C);[27] IR (KBr) (ν_{max} cm^{-1}): 3393, 3354, 1594, 1533, 1504, 1328, 1302, 1257, 1164, 1139, 1110, 946, 876, 828, 807, 745, 675; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.51–7.48 (m, 2H), 6.79 (d, *J* = 8.6 Hz, 1H), 6.34 (s, 1H), 5.27 (s, 2H), 4.17 (q, *J* = 9.6 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 140.9, 138.2, 135.1, 125.5 (q, *J* = 279.4 Hz), 114.7, 108.5, 108.0, 43. 6 (q, *J* = 32.5 Hz); ^{19}F NMR (282 MHz, DMSO-d_6): δ −70.1 (s, 3F); HRMS (ESI) *m/z* calcd for $C_8H_9F_3N_3O_2^+$ $[M+H]^+$: 236.0647; found: 236.0649.

Preparation of 5-({5-nitro-2-[(2,2,2-trifluoroethyl)amino]phenyl}amino)-5-oxopentanoic acid **(4)**

Glutaric anhydride (12.55 g, 110 mmol) and compound **3** (23.52 g, 100 mmol) were dissolved in toluene (250 mL) and stirred under reflux for 2.5 h. The precipitated product was filtered through a fine filter paper and rinsed with toluene (50 mL) three times. Product **4** was obtained as a deep-yellow solid (32.03 g, 92%); m.p. 222–224 °C; IR (KBr) (ν_{max} cm^{-1}): 3398, 3249, 2925, 1717, 1674, 1601, 1545, 1526, 1505, 1330, 1308, 1258, 1161, 1131, 1114, 944, 824, 814, 746; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.18 (br s, 1H), 9.38 (s, 1H), 8.11 (d, *J* = 2.5 Hz, 1H), 7.98 (dd, *J* = 9.2 Hz, 2.5 Hz, 1H), 7.03 (d, *J* = 9.2 Hz, 1H), 6.90 (t, *J* = 6.3 Hz, 1H), 4.25–4.13 (m, 2H), 2.43 (t, *J* = 7.4 Hz, 2H), 2.29 (t, *J* = 7.2 Hz, 2H), 1.88–1.78 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.6, 171.9, 148.0, 136.8, 125.4 (q, *J* = 279.5 Hz), 123.04, 122.97, 122.4, 110.2, 43.4 (q, *J* = 32.7 Hz), 34.9, 33.4, 20.4; ^{19}F NMR (282 MHz, DMSO-d_6): δ −69.9 (s, 3F); HRMS (ESI) *m/z* calcd for $C_{13}H_{15}F_3N_3O_5^+$ $[M+H]^+$: 350.0964; found: 350.0961.

Preparation of 4-[5-nitro-1-(2,2,2-trifluoroethyl)-1H-benzo[d]imidazol-2-yl] butanoic acid **(5)**

Compound **4** (17.46 g, 50 mmol) was added to a 12.0 mol·L^{-1} HCl solution (1.5 L) in batches, and the mixture was stirred at 25 °C for 48 h. After the reaction was complete, a large amount of a faint yellow precipitate was formed from the filtrate when the pH was adjusted to 2.0–3.0 with ammonium hydroxide. The faint yellow solid was washed with ice-water (50 mL) three times. Product **5** was obtained as a faint yellow solid (13.52 g, 82%); m.p. 225–227 °C; IR (KBr) (ν_{max} cm^{-1}): 3422, 2973, 1712, 1618, 1526, 1334, 1269, 1228, 1175, 1144, 1067, 972, 836, 817, 786, 743, 639; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.17 (br s, 1H), 8.51 (d, *J* = 2.1 Hz, 1H), 8.22 (dd, *J* = 9.0 Hz, 2.2 Hz, 1H),

7.88 (d, J = 9.0 Hz, 1H), 5.44 (q, J = 9.2 Hz, 2H), 2.99 (t, J = 7.4 Hz, 2H), 2.44 (t, J = 7.2 Hz, 2H), 2.13–2.03 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.2, 159.4, 143.2, 141.5, 139.9, 124.2 (q, J=278.5 Hz), 118.2, 114.8, 111.2, 44.0 (q, J=33.7 Hz), 32.8, 25.5, 21.7;^{19}F NMR (282 MHz, DMSO-d_6): δ −69.3 (s, 3F); HRMS (ESI) *m/z* calcd for $C_{13}H_{13}F_3N_3O_4^+$ $[M+H]^+$: 332.0858; found: 332.0853.

Preparation of ethyl 4-[5-nitro-1-(2,2,2-trifluoroethyl)-1H-benzo[d]imidazol-2-yl] butanoate **(6)**

Compound **5** (16.56 g, 50 mmol) in ethanol (150 mL) was stirred at 40 °C, concentrated sulfuric acid (5 mL) was added dropwise under stirring, and the mixture was then stirred under reflux for 3.5 h. After the reaction was complete, the mixture was evaporated in vacuo to obtain a white solid. The crude product was dissolved in dichloromethane and adjusted to pH 7.0 with a saturated aqueous solution of sodium bicarbonate, filtered, and washed with distilled water (50 mL). Product **6** was obtained as a white solid (16.85 g, 94%); m.p. 75–76 °C; IR (KBr) (ν_{max} cm^{-1}): 3414, 3115, 3009, 2983, 1716, 1619, 1524, 1394, 1329, 1265, 1224, 1178, 1162, 1146, 1115, 1065, 971, 886, 834, 744, 638; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.52 (d, J=2.2 Hz, 1H), 8.23 (dd, J=9.0 Hz, 2.2 Hz, 1H), 7.90 (d, J=9.0 Hz, 1H), 5.46 (q, J=9.2 Hz, 2H), 4.06 (q, J=7.1 Hz, 2H), 3.00 (t, J=7.4 Hz, 2H), 2.52 (t, J=7.3 Hz, 2H), 2.17–2.07 (m, 2H), 1.18 (t, J=7.1 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.6, 159.2, 143.2, 141.4, 139.8, 124.2 (q, J=278.6 Hz), 118.2, 114.8, 111.2, 59.9, 44.0 (q, J=33.8 Hz), 32.7, 25.4, 21.7, 14.1; ^{19}F NMR (282 MHz, DMSO-d_6): δ −69.3 (s, 3F); HRMS (ESI) *m/z* calcd for $C_{15}H_{17}F_3N_3O_4^+$ $[M+H]^+$: 360.1171; found: 360.1175.

Preparation of ethyl 4-[5-amino-1-(2,2,2-trifluoroethyl)-1H-benzo[d]imidazol-2-yl] butanoate **(7)**

A mixture of 10% dry palladium on carbon (0.80 g) and compound **6** (17.97 g, 50 mmol) in ethanol (150 mL) was stirred at 35 °C. A total of 80% hydrazine hydrate (9.00 g) was added dropwise over 30 min under stirring, and the mixture was stirred at 50 °C for 3.0 h. After filtration, the filtrate was evaporated under reduced pressure to give a solid. Product **7** was obtained as a white solid (15.42 g, 94%); m.p. 112–114 °C; IR (KBr) (ν_{max} cm^{-1}): 3403, 3316, 3220, 2981, 1736, 1627, 1513, 1496, 1459, 1393, 1333, 1264, 1230, 1154, 1023, 969, 861, 832, 655, 613; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.23 (d, J = 8.5 Hz, 1H), 6.74 (d, J=1.8 Hz, 1H), 6.57 (dd, J=8.5 Hz, 2.0 Hz, 1H), 5.11 (q, J=9.3 Hz, 2H), 4.78 (s, 2H), 4.05 (q, J=7.1 Hz, 2H), 2.81 (t, J=7.4 Hz, 2H), 2.46 (t, J=7.4 Hz, 2H), 2.07–1.98 (m, 2H), 1.18 (t, J=7.1 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.7, 153.9, 144.4, 143.3, 127.6, 124.5 (q, J=279.1 Hz), 111.6, 110.2, 102.2, 59.8, 43.6 (q, J=33.5 Hz), 32.9, 25.1, 22.2, 14.1; ^{19}F NMR (282 MHz, DMSO-d_6): δ −69.4 (s, 3F); HRMS (ESI) *m/z* calcd for $C_{15}H_{19}F_3N_3O_2^+$ $[M+H]^+$: 330.1429; found: 330.1423.

Preparation of ethyl 4-{5-[bis(2-hydroxyethyl) amino]-1-(2,2,2-trifluoroethyl)-1H-benzo[d] imidazol-2-yl}butanoate **(8)**

Compound **7** (6.59 g, 20 mmol) was added to a mixture of glacial acetic acid (50 mL) and water (50 mL) and stirred at 5 °C. Oxriane (5 mL) was added dropwise under stirring and the mixture was stirred at 5 °C for 24 h in a closed system. After removing the solvent under reduced pressure, the residue was dissolved in dichloromethane, washed with distilled water, and dried over anhydrous Na_2SO_4. The solvent was concentrated in vacuo, and the crude product was adjusted to pH 7.0 with a saturated aqueous solution of sodium bicarbonate, filtered, and washed with distilled water (50 mL). Product **8** was obtained as a white solid (7.48 g, 90%); m.p. 115–116 °C; IR (KBr) (ν_{max} cm^{-1}): 3286, 2989, 2955, 2876, 1741, 1628, 1591, 1501, 1450, 1419, 1390, 1289, 1260, 1189, 1168, 1058, 991, 826, 788, 654; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.15 (d, J=8.8 Hz, 1H), 7.03 (d, J=1.8 Hz, 1H), 6.80 (dd, J=8.9 Hz, 2.00 Hz, 1H), 4.63 (q, J=8.4 Hz, 2H), 4.30 (s, 2H), 4.11 (q, J=7.1 Hz, 2H), 3.83 (t, J=4.7 Hz, 4H), 3.55 (t, J=4.7 Hz, 4H), 2.86 (t, J=7.5 Hz, 2H), 2.47 (t, J=6.8 Hz, 2H), 2.22–2.13 (m, 2H), 1.24 (t, J=7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.3, 154.4, 145.3, 143.5, 128.0, 123.6 (q, J=279.0 Hz), 111.8, 109.7, 103.0, 60.6, 60.5, 56.1, 45.0 (q, J=35.5 Hz), 33.1, 26.0, 22.4, 14.3; ^{19}F NMR (282 MHz, $CDCl_3$): δ −70.3 (s, 3F); HRMS (ESI) *m/z* calcd for $C_{19}H_{27}F_3N_3O_4^+$ $[M+H]^+$: 418.1954; found: 418.1959.

Preparation of ethyl 4-{5-[bis(2-chloroethyl) amino]-1-(2,2,2-trifluoroethyl)-1H-benzo[d] imidazol-2-yl}butanoate **(9)**

A mixture of $POCl_3$ (20 mL) and compound **8** (4.17 g, 10 mmol) was stirred at 80 °C for 8 h. After removing the solvent under reduced pressure, the residue was dissolved in dichloromethane, washed with a saturated aqueous solution of sodium bicarbonate, and dried over anhydrous Na_2SO_4. The solvent was concentrated in vacuo, and the crude product was purified by column chromatography (PE: EtOAc = 30: 1 *v/v*) to provide the pure compound. Product **9** was obtained as a white solid (3.58 g, 79%); m.p. 68–69 °C; IR (KBr) (ν_{max} cm^{-1}): 3421, 2982, 2967, 1720, 1629, 1592, 1523, 1493, 1447, 1359, 1326, 1266, 1228, 1180, 1154, 1111, 1024, 981, 834, 793, 757, 734, 657, 632; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.22 (d, J=8.8 Hz, 1H), 7.08 (d, J=2.3 Hz, 1H), 6.79 (dd, J=8.9 Hz, 2.4 Hz, 1H), 4.66 (q, J=8.4 Hz, 2H), 4.13 (q, J=7.1 Hz, 2H), 3.77–3.72 (m, 4H), 3.67–3.61 (m, 4H), 2.91 (t, J=7.5 Hz, 2H), 2.51 (t, J=6.8 Hz, 2H), 2.29–2.20 (m, 2H), 1.25 (t, J=7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.2, 154.7, 143.9, 143.1, 128.5, 123.5 (q, J=278.8 Hz), 110.8, 110.0, 103.0, 60.5, 54. 6, 45.0 (q, J=35.6 Hz), 40.6, 33.1, 26.0, 22.2, 14.2; ^{19}F NMR (282 MHz, $CDCl_3$): δ −70.3 (s, 3F); HRMS

Table 2. Crystallographic characteristics, experimental and refinement data for compound **10**.

Empirical formula	$C_{17}H_{21}C_{l3}F_3N_3O_2$
Formula weight ($g{\cdot}mol^{-1}$)	462.72
Crystal system	Monoclinic
Space group	$P2_1/c$
Z	4
a (Å)	11.1969(11)
b (Å)	13.8329(13)
c (Å)	14.2207(14)
β (°)	110.192(2)
V (Å3)	2067.2(3)
Density (calc. $g{\cdot}cm^{-3}$)	1.487
Absorption coefficient (mm^{-1})	0.488
F(000), e	952
Temperature (K)	296(2)
Crystal size (m^3)	$0.24\times0.16\times0.10$
θ range for data collection	1.94–25.01
Collection range	$-13\leqslant h\leqslant 12$ $-13\leqslant k\leqslant 16$ $-14\leqslant l\leqslant 16$
Reflections collected/unique	11262/3636
R_{int}	0.0346
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	3636/2/259
Final R indices ($I>2$sigma(I))	$R_1=0.0623$, $wR_2=0.1712$
R indices (all data)	$R_1=0.0721$, $wR_2=0.1837$
Goodness-of-fit on F^2	1.039
$\Delta\rho_{max}/\Delta\rho_{min}$ ($e{\cdot}Å^3$)	0.743/−0.723

(ESI) *m/z* calcd for $C_{19}H_{25}Cl_2F_3N_3O_2^+$ $[M+H]^+$: 454.1276; found: 454.1271.

Preparation of 4-{5-[bis(2-chloroethyl)amino]-1-(2,2,2-trifluoroethyl)-1H-benzo[d]imidazol-2-yl}butanoic acid hydrochloride **(10)**

Compound **9** (2.27 g, 5 mmol) was added to HCl solution (12.0 $mol{\cdot}L^{-1}$, 10 mL), and the mixture was stirred under reflux for 2 h. After the reaction was complete, a large amount of a white precipitate was formed from the reaction liquid. This solid was washed with distilled water (10 mL) three times. Product **10** was obtained as a white solid (1.95 g, 92%); m.p. 190–191 °C; IR (KBr) (ν_{max} cm^{-1}): 3443, 2964, 2748, 2681, 2602, 1727, 1637, 1502, 1406, 1360, 1332, 1301, 1279, 1254, 1190, 1169, 1143, 1114, 981, 836, 800, 744, 721, 654; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.20 (br s, 1H), 7.89 (d, J=9.2 Hz, 1H), 7.21 (dd, J=9.2 Hz, 1.8 Hz, 1H), 6.98 (d, J=1.7 Hz, 1H), 5.70 (q, J=8.8 Hz, 2H), 3.85–3.80 (m, 8H), 3.28 (t, J=7.6 Hz, 2H), 2.47 (t, J=7.2 Hz, 2H), 2.17–2.07 (m, 2H), ^{13}C NMR(75 MHz, DMSO-d_6): δ 173.7, 153.7, 146.0, 131.9, 124.1, 123.6 (q, J=278.1 Hz), 114.0, 113.1, 94.7, 52.3, 44.7 (q, J=33.3 Hz), 41.0, 32.7, 24.0, 21.9; ^{19}F NMR (282 MHz, $CDCl_3$): δ −68.8 (s, 3F); HRMS (ESI) *m/z* calcd for $C_{17}H_{21}Cl_2F_3N_3O_2^+$ $[M-HCl+H]^+$: 426.0963; found: 426.0968.

X-Ray structure determination

Single crystals suitable for X-ray diffraction were selected, glued on fiberglass, and mounted on a Bruker APEX II CCD diffractometer equipped with a graphite-monochromatic Mo-*K*α radiation at 296 K (λ=0.71073 Å). Data collection and reduction were performed using the APEX2 software suite. Empirical absorption corrections were carried out using SADABS. The structure was solved by direct methods and expanded by difference Fourier techniques. The non-hydrogen atoms were refined anisotropically and the hydrogen atoms were added according to the theoretical models with U_{iso}(H)=1.2 (1.5 for methyl) U_{eq}(C). The structure was refined by full-matrix least-squares method on F^2 with SHELXL-97. The crystallographic data for the compound **10** (CCDC, 1827727) is summarized in Table 2.

Declaration of conflicting interests

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Supplemental material

Supplemental material (^{1}H, ^{13}C and ^{19}F NMR spectra of compound **2–10**) for this article is available online.

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