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ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 2198–2205
Bioorganic & Medicinal Chemistry

Synthesis and evaluation as MRI probe of the trifluoromethylated *p*-boronophenylalanine and its alcohol derivative

Yoshihide Hattori,[a] Hitoshi Yamamoto,[b] Hideya Ando,[c,d] Hirofumi Kondoh,[c,d] Tomoyuki Asano,[c,d] Mitsunori Kirihata,[c,d] Yoshihiro Yamaguchi[a] and Tateaki Wakamiya[a,*]

[a]*Faculty of Science and Technology, Kinki University, Higashi-osaka, Osaka 577-8502, Japan*
[b]*Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan*
[c]*Bio-Research Inc., Kobe, Hyogo 650-0047, Japan*
[d]*Graduate School of Life & Environmental Sciences, Osaka Prefecture University, Sakai, Osaka 599-8231, Japan*

Received 31 October 2006; revised 6 December 2006; accepted 7 December 2006
Available online 10 January 2007

Abstract—Boron-neutron capture therapy (BNCT) and magnetic resonance imaging (MRI) are quite attractive techniques for treatment and diagnosis of cancer, respectively. In order to develop practical tools for BNCT and MRI, novel compounds containing both the trifluoromethyl group and ^{10}B atom in a single molecule were designed. In the present study, *p*-boronophenylalanine and *p*-boronophenylalaninol with the trifluoromethyl group were synthesized, and ^{19}F NMR measurements of these compounds were carried out.

1. Introduction

Magnetic resonance imaging (MRI) is commonly used as a technique for diagnosis of cancer.[1] The characteristics of MRI are its relatively high resolution, the use of probes (or contrast enhancers) that can be stored for a long term, and the circumvention of danger resulting from radiation exposure.

In particular, MRI based on the measurement of ^{19}F atom is becoming a remarkable method for diagnosis of various diseases. For example, Higuchi et al. reported that the intravenously administered ^{19}F-containing styrylbenzene derivative labels brain plaques and allows them to be visualized in living amyloid β precursor protein (APP) transgenic mice by ^{19}F MRI.[2]

From the standpoint of treatment for brain cancer or melanoma, the boron neutron capture therapy (BNCT) based on the interaction between ^{10}B isotope and neutron has been highly noted in recent years as a quite useful technique for treatment of cancer.[3] β-[4-(^{10}B)Boronophenyl]alanine (^{10}Bpa)[4] (**1**) and β-[4-(^{10}B)boronophenyl]alaninol (^{10}Bpa-ol)[5] (**2**), in which each boron atom is enriched with ^{10}B isotope, had been created as novel ^{10}B carriers for BNCT.

In order to create novel materials to use practically for diagnosis and treatment of cancer by means of MRI and BNCT, respectively, we had already synthesized the compounds containing both fluorine[6] and ^{10}B atoms in a single molecule such as DL-β-[4-(^{10}B)borono-2,6-difluorophenyl]alanine [DL-$^{10}Bpa(2,6F_2)$] (**3**) or DL-3-[4-(^{10}B)borono-2,6-difluorophenyl]alaninol [DL-$^{10}Bpa(2,6F_2)$-ol] (**4**).[7] As far as we examined, the incorporated amounts of these compounds into C6 (rat glioma), KB (human melanoma), and HeLa (human ephithelioma) cells are almost the same as that of ^{10}Bpa being clinically used for BNCT at present.

In the present study we newly designed and synthesized DL-β-[4-(^{10}B)borono-2-trifluoromethylphenyl]alanine [DL-$^{10}Bpa(2CF_3)$] (**5**) and DL-3-[4-(^{10}B)borono-2-trifluoromethylphenyl]alaninol [DL-$^{10}Bpa(2CF_3)$-ol] (**6**), in which the fluorine atoms are increased in number compared to difluoro derivatives **3** and **4** to raise the sensitivity of detection by ^{19}F NMR (Fig. 1).

Keywords: Magnetic resonance imaging; Boron-neutron capture therapy; β-[4-(^{10}B)borono-2,6-trifluoromethylphenyl]alanine; 3-[4-(^{10}B)-borono-2,6-trifluoromethylphenyl]alaninol.
* Corresponding author. E-mail: wakamiya@chem.kindai.ac.jp

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doi:10.1016/j.bmc.2006.12.043

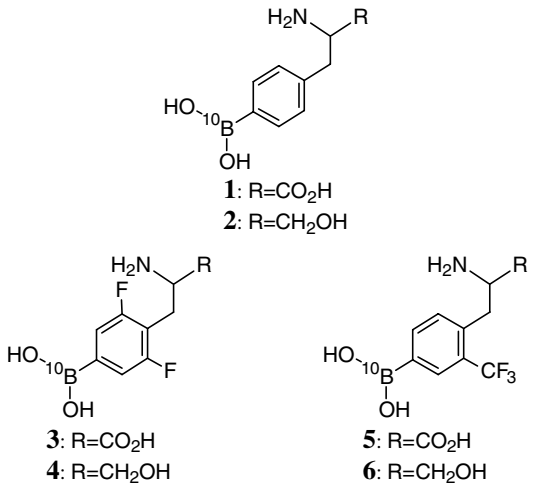


Figure 1. ^{10}Bpa (**1**) and the related compounds **2**–**6**.

2. Chemistry

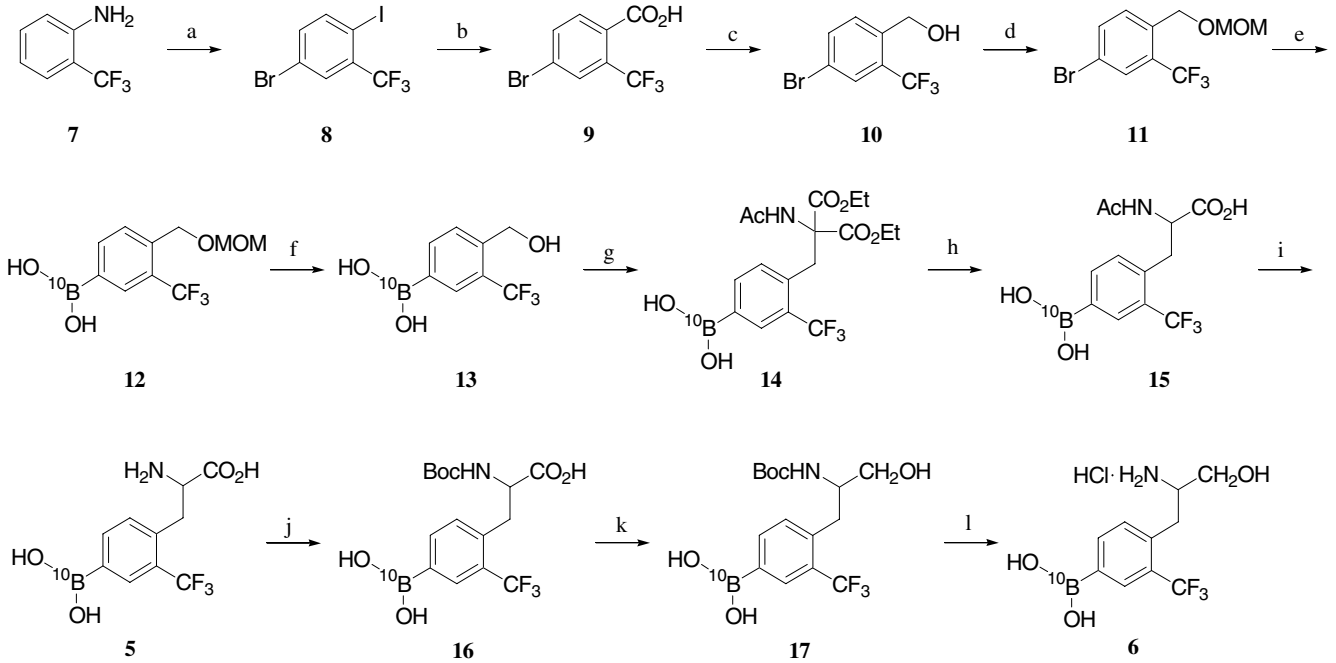
The synthesis of DL-^{10}Bpa(2CF$_3$) was carried out by the conventional method based on the reaction of alkyl halide with sodium diethyl acetamidomalonate[8] as shown in Scheme 1.

2-Aminobenzotrifluoride (**7**) was brominated according to the method reported by Roche et al.,[9] and the amino group was converted into 5-bromo-2-iodobenzotrifluoride (**8**) with *t*-BuNO$_2$ and I$_2$. The benzotrifluoride derivative **8** was reacted with *i*-PrMgCl and then CO$_2$ to give 4-bromo-2-trifluoromethylbenzoic acid (**9**). The benzoic acid derivative **9** was converted into the benzylalcohol derivative **10**, and then the alcohol group was protected with the MOM[10] group to give the methoxymethyl ether derivative **11**.

In the next step, the phenylboric acid derivative **12** was first tried to prepare from the compound **11** by the halogen-metal exchange reaction with *i*-PrMgCl and subsequent addition of ^{10}B(OMe)$_3$ in a similar manner as reported previously for the preparation of DL-^{10}Bpa(2,6F$_2$).[7] Although, the bromo group was not reacted with *i*-PrMgCl in the present case, the desired boronation was successfully achieved via lithiation of **11** with *n*-BuLi.

Treatment of **12** with 3 M HCl to cleave the MOM group gave the benzyl alcohol derivative **13**. The hydroxyl group in the compound **13** was changed to bromide with PBr$_3$, and the brominated product was coupled with sodium diethyl acetamidomalonate.[11] The diester moiety of the compound **14** was hydrolyzed and decarboxylated to give N^{α}-Ac-DL-^{10}Bpa(2CF$_3$)-OH (**15**) that was hydrolyzed with 3 M HCl to give DL-^{10}Bpa(2CF$_2$) (**5**).

The α-amino group of **5** was first protected with the Boc group, and the carboxyl group of the product **16** was then reduced via methyl ester[12] to provide the alcohol derivative **17**. The Boc group of **17** was finally cleaved with 4 M HCl/AcOEt to give DL-^{10}Bpa(2CF$_3$)-ol (**6**) as HCl salt.



Scheme 1. Reagents and conditions: (a) 1—KBr, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, $NaBO_3{\cdot}4H_2O$, AcOH, rt, 6 h; 2—*t*-BuNO$_2$, I$_2$, MeCN, rt, 16 h, 91.4%; (b) 1—*i*-PrMgCl, THF, −20 °C, 2 h; 2—CO$_2$, −20 °C, 1 h, 67.8%; (c) 1—ClCO$_2$CH(CH$_3$)$_2$, Et$_3$N, THF, 1.5 h, −10 °C; 2—NaBH$_4$, H$_2$O, 0 °C, 8 h, 97.5%; (d) MOM–Cl, DIEA, CH$_2$Cl$_2$, reflux, 24 h, 86.2%; (e) 1—*n*-BuLi, THF, −78 °C, 1 h; 2—^{10}B(OMe)$_3$, −78 °C then rt, 5 h, 71.3%; (f) 3 M HCl, THF, 60 °C, 8 h; 90.5%; (g) 1—PBr$_3$, Et$_2$O, 0 °C, 4 h; 2—sodium diethyl acetamidomalonate, THF, rt, 16 h, 79.9%; (h) 1—1 M NaOH, 80 °C, 8 h; 2—3 M HCl, 80 °C 5 h, 86.8%; (i) 1—3 M HCl, 80 °C, 24 h; 2—propylene oxide, *i*-PrOH, rt, 6 h, 83.8%; (j) (Boc)$_2$O, Na$_2$CO$_3$, acetone, H$_2$O, rt, 7 h, 90.8%; (k) 1—MeI, KHCO$_3$, rt, 24 h; 2—NaBH$_4$, LiCl, THF, MeOH, 0 °C then rt, 2 h, 91.1%; (l) 4 M HCl/AcOEt, rt, 30 min, 92.2%.

3. Results and discussion

In order to apply the compounds **5** and **6** to ^{19}F MRI, we first examined detection-sensitivity in the measurement of ^{19}F NMR of these fluorinated compounds in deuterium saline[13] (2.4, 0.24, 0.024, and 0.0024 mM). As shown in Figures 2 and 3, ^{19}F signals of the trifluoromethylated compounds **5** and **6** are detectable much more easily than those of difluorinated derivatives **3** and **4**.

From the standpoint of future MRI study, we next examined whether these fluorinated *p*-boronophenylalanine derivatives incorporated into cancer cells are detectable by ^{19}F NMR measurement. For this purpose, Ihara cells (human melanoma)[14] incorporating the compounds **3** and **5** were loaded into micro NMR tube and then subjected to the measurement of ^{19}F NMR (Fig. 4).

In the measurement of compound **3** incorporated in Ihara cells, ^{19}F signal is detectable even by four integrated counts (ca. 1 min). When integrated counts were increased to 64 (ca. 10 min) (Fig. 4a), an extra small signal was observed beside a major one, though the reason is not clarified. In the case of the measurement of compound **5**, two major signals were detected even by four integrated counts (Fig. 4b). However, detailed assignments of these two peaks are not achieved yet.

From the elucidations mentioned above, the following results were given, that is, (i) all fluorinated DL-^{10}Bpa derivatives may be usable as ^{19}F MRI probe, (ii) the carboxyl type compound is more sensitive than the corresponding alcohol derivative in ^{19}F NMR measurement, and (iii) the trifluoromethylated compound **5** is better than the compound **3** as a ^{19}F MRI probe.

Biological evaluation of the trifluoromethylated compounds **5** and **6** as boron carrier is currently being undertaken, and the results will be reported soon elsewhere. Furthermore, we are planning to apply these compounds to ^{19}F MRI for diagnosis of melanoma.

4. Experimental

4.1. General

All of the melting points are uncorrected and were measured by Yanaco MP-J3 (Yanaco CO., Ltd, Kyoto,

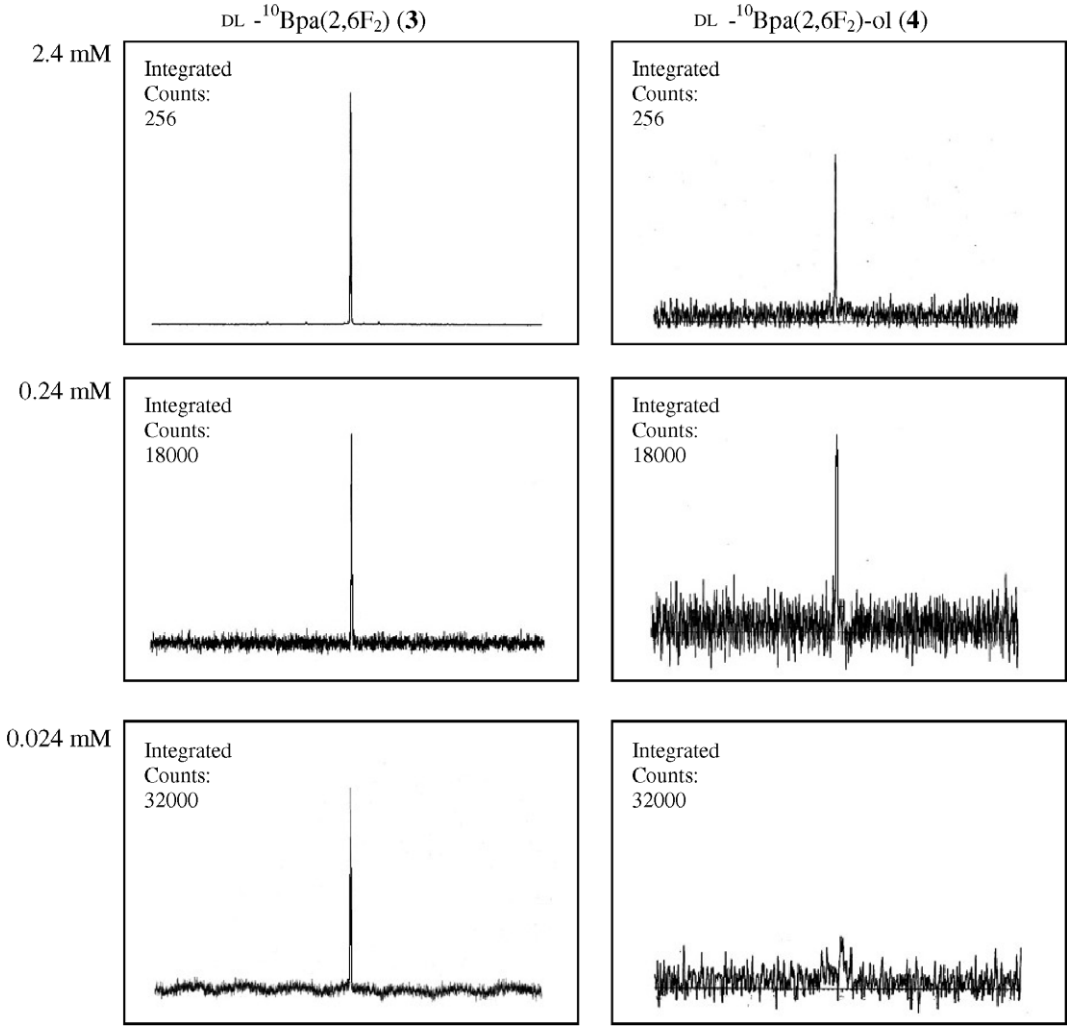


Figure 2. ^{19}F NMR spectra of compounds **3** and **4**.

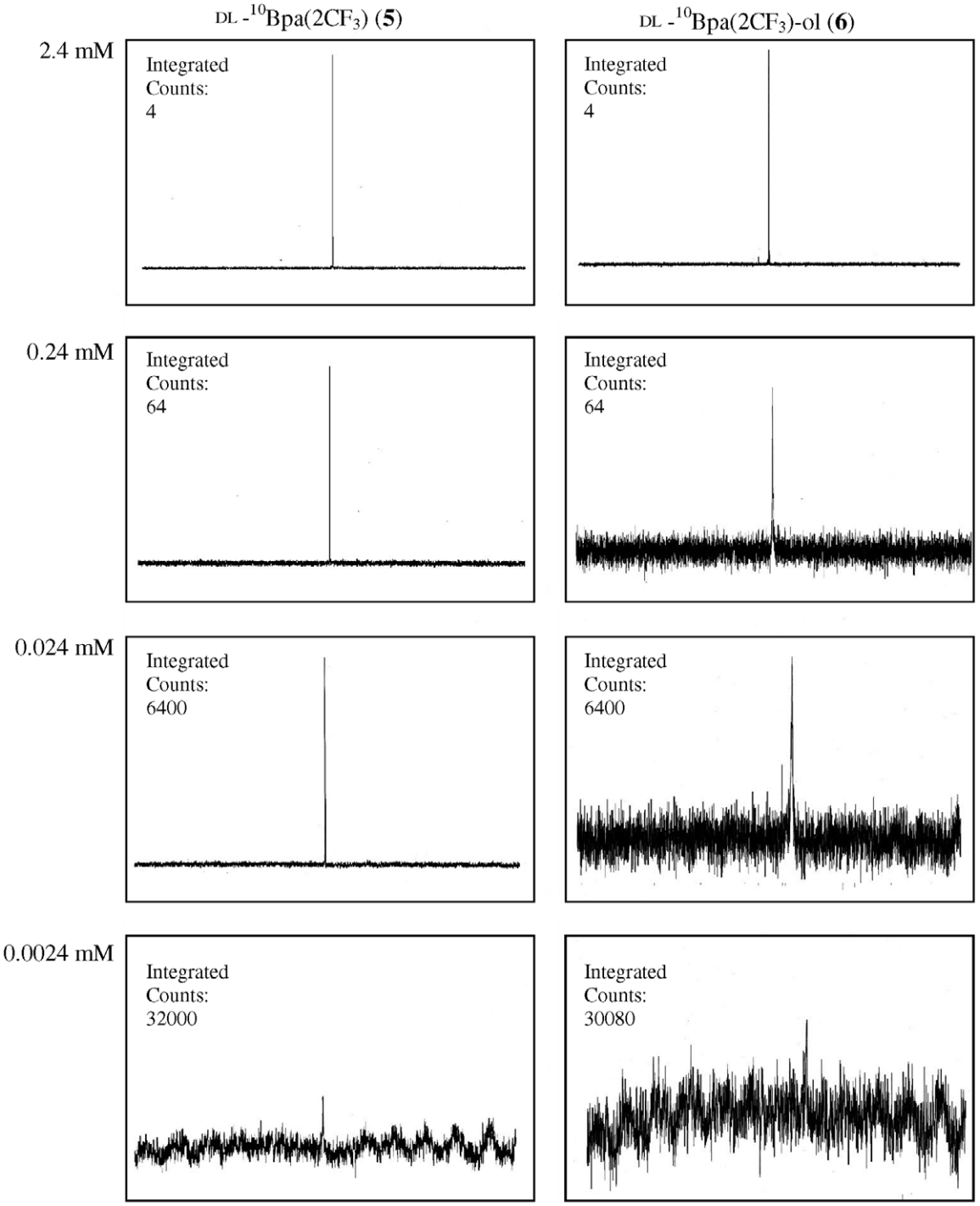


Figure 3. ^{19}F NMR spectra of compounds **5** and **6**.

Japan). Silica-gel column chromatography was carried out with silica gel PSQ100B (Fuji Silysia Chemical Ltd, Aichi, Japan). ^{1}H NMR spectra were measured on a Varian Mercury 300 [300 MHz, Varian CO., Ltd, USA] spectrometer. The chemical shifts in ^{1}H NMR are given in δ values from TMS used as the internal standard. ^{19}F NMR spectra were measured on a Varian Inova 600 [564 MHz, Varian Co., Ltd, USA] spectrometer. Matrix-assisted laser desorption ionization time of flight mass spectra (MALDI-TOF MS) were obtained on a KRATOS KOMPACT MALDI IV mass spectrometer (Shimadzu Co. Ltd, Kyoto, Japan). Measurement of high resolution mass was carried out by fast-atom bombardment mass spectrometry (FAB-MS) using a JEOL JMS-700 TKM mass spectrometer (JEOL Co. Ltd, Tokyo, Japan). Elemental analyses were performed at the MICRO CORDER JM10 (J-Science Lab Co. Ltd, Kyoto, Japan). $^{10}B(OMe)_3$ was purchased from Stella Chemifa Corporation (Osaka, Japan).

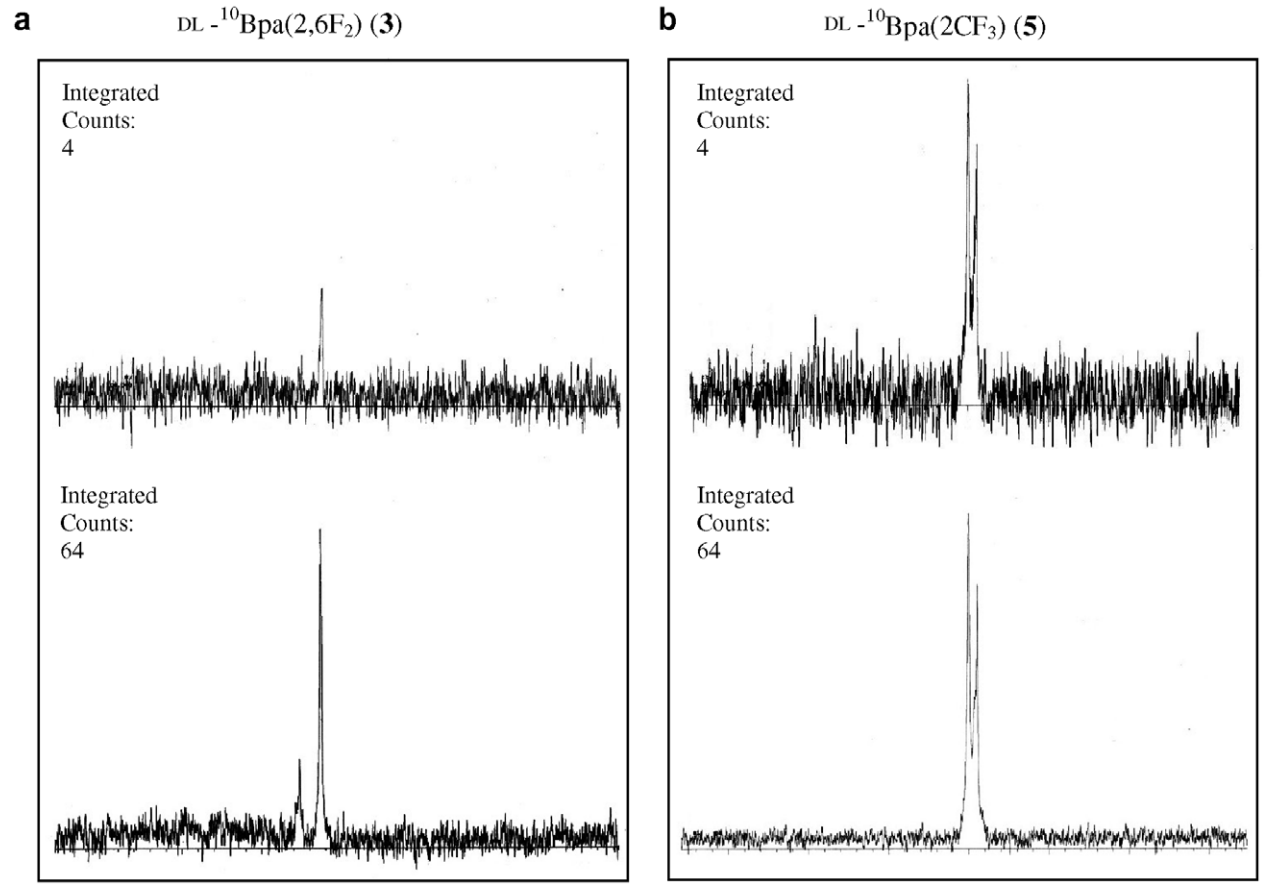


Figure 4. ^{19}F measurements of compounds **3** and **5** incorporated into Ihara cells.

4.2. Synthesis

4.2.1. 4-Bromo-2-iodobenzotrifluoride (8). To a solution of 2-aminobenzotrifluoride (1.37 g, 8.50 mmol) in AcOH (10 mL) were added KBr (1.20 g, 10.1 mmol), $NaBO_3 \cdot 4H_2O$ (1.43 g, 9.30 mmol), and $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (18.7 mg, 0.08 mmol), and the mixture was stirred for 3 h at room temperature. The reaction mixture was basified with saturated Na_2CO_3 aq and extracted with AcOEt (3× 50 mL). The combined extracts were washed with saturated $NaHCO_3$ aq (3× 40 mL) and brine (3× 40 mL), and dried over anhydrous $MgSO_4$. The dried extract was concentrated in vacuo, and the residue was dissolved in MeCN (5 mL). The solution was added to a solution of I_2 (6.47 g, 25.5 mmol) and 90% *tert*-butyl nitrite (1.47 g, 12.8 mmol) in MeCN (5 mL). After stirring for 16 h at room temperature, the reaction mixture was poured into 10% Na_2SO_3 aq (50 mL), and extracted with CH_2Cl_2 (3× 30 mL). The combined extracts were washed with 10% Na_2SO_3 aq (3× 30 mL), H_2O (3× 30 mL) and brine (3× 30 mL), and dried over anhydrous $MgSO_4$. The dried extract was concentrated in vacuo, and the residual solid was recrystallized from hexane to give **8** as yellow crystals (2.73 g, 91.4%): mp 125–129 °C. ^1H NMR ($CDCl_3$) δ 7.34 (dd, 1H, J = 2.4 Hz, 8.1 Hz), 7.77 (d, 1H, J = 2.4 Hz), 7.87 (d, 1H, J = 8.1 Hz); Anal. Calcd for $C_7H_3BrF_3I$: C, 23.96; H, 0.86. Found: C, 23.42; H, 1.04.

4.2.2. 4-Bromo-2-trifluoromethylbenzoic acid (9). To a solution of **8** (18.6 g, 53.0 mmol) in anhydrous THF (50 mL) was added *i*-PrMgCl (2.0 M solution in THF, 26.5 mL, 53.0 mmol) dropwise at −20 °C under Ar atmosphere. After stirring for 2 h, CO_2 gas was bubbled into the reaction mixture for 1 h under cooling at −20 °C. To the reaction mixture was added saturated NH_4Cl aq (100 mL) and extracted with AcOEt (3× 60 mL). The combined extracts were dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residual solid was recrystallized from AcOEt and hexane to give **9** as dark yellowish brown crystals (9.50 g, 66.4%): mp 113–117 °C. ^1H NMR ($CDCl_3$) δ 7.81 (d, 1H, J = 8.1 Hz), 7.89 (d, 1H, J = 8.1 Hz), 7.96 (s, 1H); Anal. Calcd for $C_7H_3BrF_3I$: C, 23.96; H, 0.86. Found: C, 23.42; H, 1.04.

4.2.3. 4-Bromo-2-trifluoromethylbenzyl alcohol (10). To a solution of **9** (3.47 g, 12.9 mmol) and Et_3N (1.96 g, 19.4 mmol) in anhydrous THF (50 mL) was added $ClCOOCH(CH_3)_2$ (2.38 g, 19.4 mmol) dropwise over a 10-min period at −20 °C. After stirring for 1.5 h at −10 °C, to the reaction mixture was added ice-cooled water (50 mL), followed by an addition of $NaBH_4$ (2.44 g, 64.5 mmol) in limited amounts at 0 °C. After stirring for 8 h at room temperature, the reaction mixture was acidified with citric acid and diluted with H_2O (50 mL) to dissolve precipitated inorganic materials. After evaporation of THF, the aqueous solution was extracted with AcOEt (3× 40 mL). The combined extracts were washed with saturated $NaHCO_3$ aq (3× 30 mL) and brine (3× 30 mL), and dried over anhydrous

$MgSO_4$. The dried extract was concentrated in vacuo, and the residue was purified by silica-gel column chromatography (silica gel: 90 g, hexane/AcOEt = 3:1) to give **10** as colorless oil (3.21 g, 97.5%): ^{1}H NMR ($CDCl_3$) δ 1.97 (t, 1H, J = 5.7 Hz), 4.84 (d, 2H, J = 5.7 Hz), 7.62 (d, 1H, J = 8.4 Hz), 7.71 (d, 1H, J = 8.4 Hz), 7.77 (s, 1H).

4.2.4. 4-Bromo-2-trifluoromethylbenzyl methoxymethyl ether (11). To a solution of **10** (2.77 g, 10.9 mmol) in CH_2Cl_2 (50 mL) were added DIEA (3.53 g, 27.3 mmol) and MOM–Cl (1.76 g, 21.8 mmol), and the reaction mixture was refluxed for 24 h. After evaporation of the solvent, the residue was dissolved in AcOEt (50 mL). The solution was washed with 10% aqueous citric acid (3× 20 mL), saturated $NaHCO_3$ (3× 20 mL) and brine (3× 20 mL), and dried over anhydrous $MgSO_4$. The dried organic layer was concentrated in vacuo, and the residue was purified by silica-gel column chromatography (silica gel: 60 g, hexane/AcOEt = 9:1) to give **11** as colorless oil (2.81 g, 86.2%): ^{1}H NMR ($CDCl_3$) δ 3.42 (s, 3H), 4.72 (s, 2H), 4.75 (s, 2H), 7.58 (d, 2H, J = 8.4 Hz), 7.68 (d, 2H, J = 8.4 Hz), 7.78 (s, 1H).

4.2.5. 4-(^{10}B)Borono-2-trifluoromethylbenzyl methoxymethyl ether (12). To a solution of **11** (2.54 g, 8.49 mmol) in anhydrous THF (30 mL) was added a solution of 1.6 M *n*-BuLi in hexane (5.57 mL, 8.91 mmol) at −78 °C under Ar atmosphere. After stirring for 1 h at −78 °C, to the reaction mixture was added $^{10}B(OMe)_3$ (1.31 g, 12.7 mmol), and the solution was stirred for 5 h at −78 °C. To the reaction mixture was added saturated NH_4Cl aq (30 mL) at room temperature, and the mixture was extracted with Et_2O (3× 60 mL). The combined extracts were washed with 10% aqueous citric acid (3× 60 mL) and brine (3× 60 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by silica-gel column chromatography (silica gel: 60 g, hexane/AcOEt = 1:1) to give **12** as colorless oil (1.59 g, 71.3%): ^{1}H NMR (CD_3COCD_3) δ 3.36 (s, 3H), 4.74 (s, 2H), 4.77 (s, 2H), 7.49 (s, 2H), 7.75 (d, 1H, J = 7.8 Hz), 8.12 (d, 1H, J = 7.8 Hz), 8,18 (s, 1H).

4.2.6. 4-(^{10}B)Borono-2-trifluoromethylbenzyl alcohol (13). The solution of **12** (1.57 g, 5.96 mmol) in THF (30 mL) and 3 M HCl (30 mL) was stirred for 8 h at 60 °C. The reaction mixture was extracted with Et_2O (3× 40 mL), and the combined extracts were washed with water (3× 50 mL) and brine (3× 50 mL). The organic layer was dried over $MgSO_4$, and the solution was concentrated in vacuo. The residue was purified by silica-gel column chromatography (silica gel: 50 g, CH_2Cl_2/MeOH = 9:1) to give **13** as colorless crystals (1.18 g, 90.5%): mp 289–294 °C. ^{1}H NMR (CD_3COCD_3) δ 4.77 (s, 2H), 7.58 (s, 2H), 7.71 (d, 1H, J = 7.8 Hz), 8.11 (d, 1H, J = 7.8 Hz), 8.18 (s, 1H). Anal. Calcd for $C_8H_8{}^{10}BF_3O_3$: C, 43.84; H, 3.68. Found: C, 43.45; H, 3.93.

4.2.7. 2-Acetylamino-2-[4-(^{10}B)borono-2-trifluoromethylphenyl]methylmalonic acid diethyl ester (14). To a solution of **13** (1.00 g, 4.56 mmol) in Et_2O (50 mL) was added slowly PBr_3 (1.48 g, 5.47 mmol) at 0 °C, and the mixture was stirred for 4 h at 0 °C. To the reaction mixture was added ice-cooled water (50 mL), and Et_2O layer was once taken out. The aqueous layer was extracted with Et_2O (3× 40 mL). The combined Et_2O layer and extracts were washed with water (3× 50 mL) and brine (3× 50 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residual solid was dissolved in anhydrous DMSO (5 mL), and the solution was added to a suspension of sodium diethyl acetamidomalonate[8] (1.31 g, 5.47 mmol) in anhydrous DMSO (5 mL) under Ar atmosphere. After stirring for 6 h, the reaction mixture was acidified with 1 M HCl and extracted with AcOEt (3× 30 mL). The combined extracts were washed with saturated $NaHCO_3$ (3× 20 mL) and brine (3× 20 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crystalline residue was recrystallized from AcOEt and hexane to give **14** as colorless crystals (1.52 g, 79.9%): mp 131–134 °C. ^{1}H NMR (CD_3COCD_3) δ 1.17 (t, 6H, J = 7.4 Hz), 2.00 (s, 3H), 3.85 (s, 2H), 4.00–4.22 (m, 4H), 7.37 (d, 1H, J = 7.8 Hz), 7.51 (s, 2H), 7.99 (d, 1H, J = 7.8 Hz), 8.14 (s, 1H). Anal. Calcd for $C_{17}H_{21}{}^{10}BF_3NO_7$: C, 48.81; H, 5.06; N, 3.35. Found: C, 48.43; H, 5.15; N, 3.14.

4.2.8. N^{α}-Acetyl-DL-β-[4-(^{10}B)borono-2-trifluoromethylphenyl]alanine (15). A mixture of **14** (1.00 g, 2.39 mmol) in 1 M NaOH (40 mL) was stirred for 8 h at 80 °C. To the cooled reaction mixture was added 3 M HCl (20 mL), and the solution was stirred for 4 h at 80 °C. The reaction mixture was extracted with AcOEt (3× 40 mL), and the combined extracts were washed with brine (3× 30 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crystalline residue was recrystallized from AcOEt and hexane to give **15** as colorless crystals (660 mg, 86.8%): mp 158–162 °C. ^{1}H NMR (CD_3COCD_3) δ 1.87 (s, 3H), 3.08–3.48 (m, 2H), 4.78–4.86 (m, 1H), 7.51–7.58 (m, 4H), 8.01 (d, 1H, J = 6.0 Hz), 8.18 (s, 1H); MALDI-TOF MS: found m/z 319.2 $[M+H]^+$ (calcd for $C_{12}H_{13}{}^{10}BF_3NO_5$+H: 319.1); Anal. Calcd for $C_{12}H_{13}{}^{10}BF_3NO_5$: C, 45.29; H, 4.12. N, 4.40; Found: C, 45.64; H, 4.38; N. 4.09.

4.2.9. DL-β-[4-(^{10}B)Borono-2-trifluoromethylphenyl]alanine (5). A mixture of **15** (588 mg, 1.85 mmol) in 3 M HCl was stirred for 12 h at 80 °C. The reaction mixture was washed with Et_2O (3× 30 mL) and concentrated in vacuo. The residue was dissolved in *i*-PrOH (10 mL), and to the solution was added propylene oxide (215 mg, 3.70 mmol). After stirring for 24 h, the reaction mixture was concentrated in vacuo. The crystalline residue was recrystallized from water to give **5** as colorless crystals (428 mg, 83.8%): mp 295–300 °C (dec). ^{1}H NMR (D_2O) δ 2.63–2.88 (m, 2H), 3.66 (t, 1H, J = 7.8 Hz), 6.81 (d, 1H, J = 7.4 Hz), 7.22 (d, 1H, J = 7.4 Hz), 7.35 (s, 1H); MALDI-TOF MS: found m/z 277.3 $[M+H]^+$ (calcd for $C_{10}H_{11}{}^{10}BF_3NO_4$+H: 277.1); Anal. Calcd for $C_{10}H_{11}{}^{10}BF_3NO_4$+$2H_2O$: C, 38.47; H, 4.84; N, 4.49; Found: C, 38.23; H, 5.04; N. 4.40.

4.2.10. N^{α}-*tert*-Butoxycarbonyl-DL-β-[4-(^{10}B)borono-2-trifluoromethylphenyl]alanine (16). To a solution of **5** (200 mg, 0.724 mmol) in water (10 mL) and acetone

(10 mL) were added Na_2CO_3(84.3 mg, 0.796 mmol) and Boc_2O (174 mg, 0.796 mmol), and the mixture was stirred for 12 h. The reaction mixture was acidified with 10% aqueous citric acid, and acetone was removed by evaporation in vacuo. The aqueous solution was extracted with AcOEt (3× 40 mL), and the combined extracts were washed with 10% aqueous citric acid (3× 30 mL) and brine (3× 30 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crystalline residue was recrystallized from AcOEt and hexane to give **16** as colorless crystals (247 mg, 90.8%): mp 291–292 °C (dec). ^{1}H NMR (CD_3COCD_3) δ 1.32 (s, 9H), 3.08–3.51 (m, 2H), 4.44–4.53 (m, 1H), 6.49–6.51 (br, 1H), 7.44 (br, 2H), 7.55 (d, 1H, J = 7.8 Hz), 8.03 (d, 1H, J = 7.8 Hz) 8.17 (s, 1H); Anal. Calcd for $C_{15}H_{19}{}^{10}BF_3NO_6$: C, 47.87; H, 5.09; N, 3.72. Found: C, 48.48; H, 5.37; N, 3.66.

4.2.11. N^{α}-*tert*-Butoxycarbonyl-DL-3-[4-(^{10}B)borono-2-trifluoromethylphenyl]alaninol (17). To a solution of **16** (2.92 g, 7.76 mmol) in DMF (25 mL) were added $KHCO_3$ (1.55 g, 15.5 mmol) and MeI (2.20 g, 15.5 mmol), and the mixture was stirred for 24 h. The reaction mixture was concentrated in vacuo, and the residue was suspended in AcOEt (100 mL). The suspension was washed with 10 % citric acid (3× 30 mL), saturated $NaHCO_3$ aq (3× 30 mL) and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. To the solution of the residue in THF (50 mL) were added LiCl (1.64 g, 38.8 mmol), $NaBH_4$ (2.94 g, 77.6 mmol), and MeOH (50 mL) at 0 °C. After stirring for 6 h, to the reaction mixture was added 10% citric acid (100 mL), and the aqueous solution was extracted with AcOEt (3× 60 mL). The combined extracts were dried over $MgSO_4$ and concentrated in vacuo. The oily residue thus obtained was purified by silica-gel column chromatography (silica gel: 150 g, CH_2Cl_2/MeOH = 19:1) to give **17** as colorless amorphous solid (2.56 g, 91.1%): ^{1}H NMR (CD_3COCD_3) δ 1.30 (s, 9H), 2.88–2.99 (m, 2H), 3.15–3.35 (m, 2H), 3.55–3.66 (m, 1H), 7.40 (br, 2H), 7.41 (d, 1H, J = 7.8 Hz), 8.01 (d, 1H, J = 7.8 Hz) 8.16 (s, 1H).

4.2.12. DL-3-(4-(^{10}B)Borono-2-trifluoromethylphenyl)-alaninol (6) hydrochloride. The compound **17** (900 mg, 2.72 mmol) was dissolved in 4 M HCl/AcOEt (10 mL), and the solution was stirred for 10 min. The reaction mixture was concentrated in vacuo, and the residue was dissolved in H_2O (20 mL). The aqueous solution was washed with Et_2O (3× 10 mL) and concentrated in vacuo. The crystalline residue was recrystallized from MeOH and Et_2O to give **6** as colorless crystals (671 mg, 92.6 %): mp 211–218 °C (dec). ^{1}H NMR (D_2O) δ 2.87–2.91 (m, 2H), 3.37–3.42 (m, 2H), 3.49–3.54 (m, 1H), 7.21 (d, 1H, J = 7.8 Hz), 7.64 (d, 1H, J = 7.8 Hz), 7.79 (s, 1H); MALDI-TOF MS: found m/z 263.2 $[M+H]^+$ (calcd for $C_{10}H_{13}{}^{10}BF_3NO_3$ +H: 263.1); Anal. Calcd for $C_{10}H_{14}{}^{10}BClF_3NO_3$: C, 40.21; H, 4.72; N, 4.69. Found: C, 40.07; H, 4.96; N 4.55.

4.3. In vitro evaluation as ^{19}F MRI probe

4.3.1. Cells and cell culture[7]. Ihara (human melanoma) cell line was used in ^{19}F NMR study. Cells were cultured in Dulbecco's Minimum Essential Medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM glutamine, 24 mM sodium bicarbonate at 37 °C in a 5% CO_2 atmosphere.

Cells in mono-layer were harvested with 0.25% trypsin / 0.02% EDTA in Ca^{2+}-free phosphate-buffered saline (PBS).

4.3.2. Boron incorporation into Ihara cells[7]. Cultures were inoculated with 4.0×10^7 cells/dish, and cells were grown for 24 h in DMEM. The medium was replaced with that containing DL-^{10}Bpa(2,6F_2) (**3**) or DL-^{10}Bpa(2CF_3) (**5**) (final concentration was 2.0 mM in each case), and then the cells were cultured for 24 h.

4.3.3. ^{19}F NMR measurement of fluorinated DL-^{10}Bpa incorporated into Ihara cell. The medium was removed by aspiration, and the cells were washed twice with PBS and deuterium saline. After centrifugation, the clear supernatant was removed by aspiration, and the residual cells (250 μL) are transferred into micro NMR tube. The ^{19}F signals of fluorinated DL-^{10}Bpa incorporated into the cells were measured by ^{19}F NMR.

Acknowledgment

This work was supported in part by Grants-in-Aid for Scientific Research Nos. 11680953 and 11680678 from the Ministry of Education, Science, Sports, and Culture of Japan.

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