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A New Water-soluble p-Boronophenylalanine Derivative for Neutron Capture Therapy

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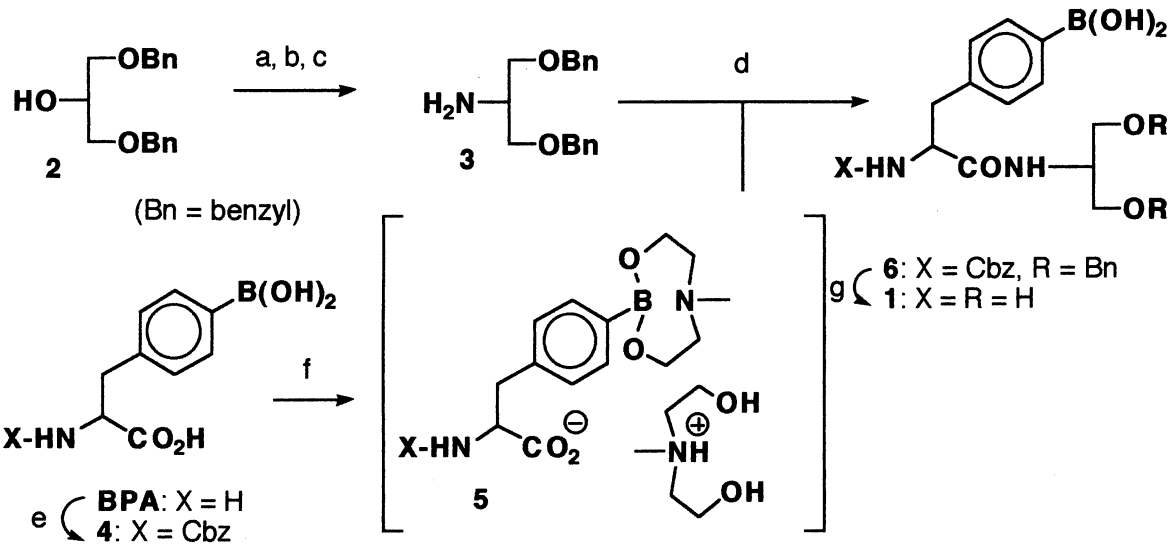
A p-boronophenylalanine (BPA) derivative, that is ca. thousand times more water-soluble and is incorporated with higher tumour/normal cell ratio than BPA itself, has been synthesized.

(±)-BPA[1] has been used in clinical level for Neutron Capture Therapy (NCT)[2] of human-melanoma cancer[3] since BPA is a kind of phenylalanine or tyrosine analog that are strongly incorporated in the cells for the production of melanin. However, BPA had been used as its hydrochloride or alkali metal salt because of the low solubility of BPA in water. Recently, the monosaccharides complexes of BPA have been used to enhance water solubility.[4] However, BPA itself seems to be easily released from the complexes *in vivo* because of the labile chemical interactions between the monosaccharides and BPA. It is desired to develop alternative water soluble BPA analogue.

We report the synthesis of the BPA derivative **1** having a tight covalent bond between BPA and the water solubilizing moiety **3**. The synthesis of **1** is shown in Scheme 1. Hydroxyl group of 1,3-dibenzylglycerol (**2**)[5] was converted to an amino group via tosylation, azide substitution and reduction procedures to give **3** in 76% overall yield. The amino group of (±)-BPA was protected with carbobenzyloxy (Cbz) group to give **4** in 98% yield. The direct condensation of **3** and **4** gave some unidentified polar materials probably because of the strong interaction between amino group of **3** and boronic moiety of **4**. When N-methyldiethanolamine[6] was used as a protection group of the boronic moiety of **4** to form **5** *in situ*, the desired compound **6** was obtained in 95% yield. The three benzyl moieties of **6** were deprotected in the presence of palladium hydroxide in ethanol/1 mol dm^{-3} hydrochloric acid to give the hydrochloride salt of **1** in 77% yield. Purification of the salt of **1** by cation-exchange resin and high performance liquid chromatography gave **1**: IR (neat) 3350, 3080, 2940, 1660, 1610, 1540, 1420, 1350, 1320, 1260, 1090, 1050, 930, 880, 810 cm^{-1}; ^{1}H-NMR (D_2O) δ 7.47 (d, J = 7.0 Hz, 2H,

aromatic), 7.06 (d, J = 7.0 Hz, 2H, aromatic), 3.67 (m, 1H, -CONH-C*H*($CH_2OH)_2$), 3.63 (t, J = 7,0 Hz, 1H, H_2N-C*H*-CONH-), 3.41 (d, J = 4.5 Hz, 2H, -CH-C*H*$_2$OH), 3.24, (dd, J = 5.0, 11.0 Hz, 1H, -CH-C*H*$_2$OH), 3,15 (dd, J = 6.0, 11.0 Hz, 1H, -CH-C*H*$_2$OH), 2.83 (dd, J = 7.0, 13.5 Hz, 1H, -C*H*$_2$-C_6H_4-), 2.79 (dd, J = 7.0, 13.5 Hz, 1H, -C*H*$_2$-C_6H_4-); ^{13}C-NMR[7] (D_2O) δ 175.6, 138.8, 134.6, 130.5, 129.9, 62.6, 61.6, 57.0, 52.9, 40.8.



a. p-$CH_3C_6H_4SO_2Cl$, pyridine b. NaN_3, dimethylformamide, 100 °C
c. $LiAlH_4$, ether d. N-hydroxybenzotriazole, C_2H_5N=C=$NCH_2CH_2CH_2NMe_2$•HCl
e. Cbz-Cl, NaOHaq f. 2 equiv. of $MeN(CH_2CH_2OH)_2$ g. $Pd(OH)_2$/C, H_2, ethanol•HClaq

Scheme 1. Synthesis of 1.

The solubility of **1** in water is 0.66M which is about thousand times more than the previously reported value of BPA (7.66×10^{-3}M).[4] The IC_{50} values of **1** were so high that 1.77×10^{-2}M of **1** was not able to kill more than 50% of the cultured B-16 human melanoma cell (B16)[8] even after three days. Under the same cultured conditions, the IC_{50} value of BPA was 8.55×10^{-3} mol dm^{-3}. Based on NCT therapeutic principle,[9] it is generally accepted that the lower the cytotoxicity is, the better the ^{10}B carrier is. Accordingly, lower toxicity of **1** is quite promising.

The measurement of the incorporation of **1** and BPA toward B16 melanoma cells was carried out by the quantitative analysis of the remaining boron atoms in the cells using inductively coupled plasma mass (ICP-

MS)[10] method (Table 1). Under the same conditions, the examination toward the cultured TIG-1-20 human baby's normal cell (T20)[8] was also carried out. Incorporation of **1** toward B16 was slightly higher than BPA (entry 2 vs. 1). In contrast, incorporation of **1** toward T20 is quite lower than BPA (entry 4 vs. 3). Five times more selective affinity toward B16 than that toward T20 was observed when **1** was used (entry 2 vs. 4) while BPA has almost no selectivity (entry 1 vs. 3).

Table 1. Incorporation Examination of **1** and BPA

Entry	Cell	Compound[a]	Incorporated boron atoms in the cell (10^{-8} mol in 10^6 cells)
1	B16	BPA	2.7
2	B16	**1**	3.7
3	T20	BPA	2.9
4	T20	**1**	0.7

a) The boron compound (0.4 mmol) in Eagle-MEM medium (5 mL) was administrated to the cultured cell (briefly 6.675×10^5) in the same medium (15 mL) at 37 °C. After 24 h, the medium was removed. The residual cells were immediately washed with PBS(-) and dissolved in 60% of NaClOaq/30% of H_2O_2aq. The mixture was heated at 70 °C until the solid disappeared.

In conclusion, we have developed a new BPA analog and demonstrated that both water solubility and boron uptake are enhanced when newly designed branched glycerol[5] is attached to BPA. Several other water-soluble BPA derivatives using the branched glycerol units are now in progress.

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