Supporting Information

Water-soluble *closo*-Docecaborate-Containing Pteroyl Derivatives Targeting Folate Receptor-Positive Tumors for Boron Neutron Capture Therapy

Tomoko Nakagawa,^a Hidehisa Kawashima,^b Taiki Morita,^{a,b} and Hiroyuki Nakamura*,^{a,b}

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^a School of Life Science and Technology, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8503, Japan.

^b Laboratory for Chemistry and Life Science, Institute of Innovative Research, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8503, Japan.

Spectral Date of PBC 1-4

NMR spectra were recorded on a BRUKER BIOSPIN AVANCE II 400 (400 MHz for ¹H) or a BRUKER BIOSPIN AVANCE III HD500 (125 MHz for ¹³C, 160 MHz for ¹¹B) in the indicated solvent. Chemical shifts are reported in units of parts per million (ppm) relative to tetramethylsilane (0.00 ppm), CDCl₃ (7.26 ppm), DMSO- d_6 (2.50 ppm), CD₃CN (1.94 ppm) or D₂O (4.79 ppm) for ¹H NMR and CDCl₃ (77.2 ppm), DMSO- d_6 (39.5 ppm) or CD₃CN (1.32 ppm) for ¹³C NMR. Boron trifluoride diethyl ether complex (BF₃·OEt₂) was used as the external standard for ¹¹B NMR spectra. Multiplicities are reported by using the following abbreviations: s; singlet, d; doublet, dd; doublet of doublets, t; triplet, tt; triplet of triplets, sext.; sextet, m; multiplet, *J*; coupling constants in Herts (Hz). IR spectrum was recorded on a JASCO Corporation FT/IR-4100 FT-IR Spectrometer. Only the strongest and/or structurally important peaks were reported as the IR data given in cm⁻¹. HRMS (ESI-TOF-MS) were measured with Bruker micrOTOF II.

PBC 1: a brown solid. ¹HNMR (400 MHz, D₂O) δ 8.66 (s, 1H), 7.64 (d, J = 8.5 MHz, 2H), 6.77 (d, J = 8.5 MHz, 2H), 4.59 (s, 2H) , 3.66-3.71 (m, 8H), 3.56 (t, 2H); ¹³CNMR (125 MHz, D₂O) δ 170.4, 150.8, 149.8, 148.4, 135.7, 129.1, 127.7, 121.9, 112.6, 71.0, 68.8, 67.6, 45.8, 39.5; ¹¹BNMR (160 MHz, D₂O) δ 6.36, -16.40, -18.28, -23.33; HRMS (ESI, negative): m/z calcd. for C₁₈H₃₁B₁₂N₇O₄Na [M+Na]⁻: 562.3541, found 562.3503; IR (KBr): 3566, 3391, 2928, 2871, 2482, 2361, 1605, 1510, 1304, 1176, 1159, 1118, 1057, 1029, 943, 837, 822, 768 cm⁻¹; mp > 300 °C.

PBC 2: a brown solid. ¹HNMR (400 MHz, D₂O) δ 8.70 (s, 1H), 7.69 (d, J = 8.3 MHz, 2H), 6.85 (d, J = 8.2 MHz, 2H), 4.65 (s, 2H) , 4.32 (t, 1H), 3.54-3.31 (m, 6H), 3.13-3.17 (m, 4H), 2.56-2.10 (m, 4H); ¹³C-NMR (125 MHz, D₂O) δ 178.8, 175.7, 169.2, 167.3, 157.9, 153.6, 150.8, 148.5, 148.3, 129.2, 129.0, 129.0, 127.4, 121.2, 112.4, 70.9, 68.3, 67.5, 55.3, 45.6, 32.7, 27.7; ¹¹BNMR (160 MHz, D₂O) δ 6.16, -16.33, -18.22, -22.9; HRMS (ESI, negative): m/z calcd. for C₂₃H₃₈B₁₂N₈O₇Na [M+Na]⁻: 691.3970, found 691.3979; IR (KBr): 3567, 3385, 2926, 2856, 2733, 2485, 2361, 1682, 1606, 1508, 1304, 1176, 1159, 1109, 1065, 1026, 951, 839, 822, 770, 723 cm⁻¹; mp > 300 °C.

PBC 3: a brown solid. ¹HNMR(400 MHz, DMSO- d_6) δ 8.47 (s, 1H), 7.51 (s, 2H), 6.61 (s, 2H), 4.37 (s, 2H), 3.58-3.45 (m, 6H), 3.35-3.27 (m, 4H), 2.64 (s, 2H); ¹³CNMR (125 MHz, DMSO- d_6) δ 173.8, 172.0, 169.8, 154.2, 151.2, 146.9, 129.2, 112.4, 71.0, 70.9, 68.6, 68.5, 67.6, 55.3, 48.9, 45.5, 43.1, 39.1, 38.7; ¹¹BNMR (160 MHz, DMSO- d_6) δ 6.12, -16.79, -17.98, -22.95; HRMS (ESI, negative): m/z calcd. for C₂₀H₃₄B₁₂N₈O₅Na

[M+Na]⁻: 619.3757, found 619.3711; IR (KBr): 3568, 3368, 2931, 2873, 2491, 2371, 1605, 1508, 1445, 1307, 1182, 1114, 1069, 943, 836, 822, 769 cm⁻¹; mp > 300 °C.

PBC 4: a brown solid. ¹HNMR(400 MHz, DMSO-*d*₆) δ 8.63 (s, 1H), 7.65 (d, *J* = 7.9 MHz, 2H), 7.68 (d, *J* = 8.0 MHz, 2H), 4.60 (s, 2H), 3.55-3.77 (m, 12H), 3.32 (t, 1H), 3.22-3.17 (m, 3H); ¹³CNMR (125 MHz, D₂O) δ 178.0, 175.9, 170.0, 160.5, 148.3, 150.6, 130.9, 129.2, 127.8, 112.5, 71.5, 68.8, 68.4, 67.6, 55.2, 50.6, 49.7, 45.8, 39.1, 38.7; ¹¹BNMR (160 MHz, D₂O) δ 5.84, -16.58, -18.45, -23.7, HRMS (ESI, negative): m/z [M+Na]⁻ calcd. for C₂₁H₃₆B₁₂N₈O₅Na [M+Na]⁻: 633.3914; found 633.3914; FT-IR (KBr): 3557, 3365, 2923, 2853, 2484, 2365, 1653, 1508, 1452, 1335, 1160, 1110, 1055, 943, 836, 820, 768 cm⁻¹; mp > 300 °C.



Figure S1. Western blot analysis of FR α in various cell lines. HeLa cell: human cervical carcinoma; MCF-7 cell: human caucasian breast adenocarcinoma; U87MG: human glioblastoma; CT26 cell: mouse colon cancer; A549: human alveolar adenocarcinoma. Overexpression of FR α was observed in HeLa, U87MG, and CT26 cells. 1×10^6 cells of HeLa, MCF-7, U-87 MG, CT26 or A549 was added 1 mL 5× SDS-PAGE sample buffer, and the mixture was boiled at 95 °C for 5 min and was then subjected to SDS-polyacrylamide gel (10 % acrylamide) electrophoresis (PAGE). Acrylamide gel which obtained by SDS-PAGE was transferred toward membrane filter at the condition of 300 mA for 13 min. Soaked and shaked in immunoblock solution for 1 h. It was washed by TBS (Tris Buffered Saline) for 10 min in 3 times. Primary antibody was added and incubated at the condition of 4 °C for 12 h. HRP-conjugated secondary antibody response at the condition of r.t. for 1 h after washed by TTBS buffer for 10 min in 3 times. The chemi luminescence intensity was measured with imager after added detection reagent.



Figure S2. Quantitative analysis of folate receptor (FR α) expressed in HeLa, MCF-7, U87MG, CT26, and A549 cells by flow cytometry using anti-FR α antibody. 5×10^5 cells of HeLa, MCF-7, U-87 MG, CT26 or A549 were washed with 1 mL PBS and then added immunoblock solution. After incubating it for 15 min at 4 °C, the solution was centrifuged (3 min, 2000 rpm). Then supernatant was removed and primary antibody (20 µg/mL in PBS) was added. After incubating it for 30min at 4°C, the solution was centrifuged and the supernatant was removed. After washed with PBS in three times, FITC-conjugated secondary antibody (2 µg/mL in PBS) was added. After supernatant was removed and washed with PBS in three times. And this cell pellet was dissolved with 500 µL PBS. Fluorescence signals were observed using a flow cytometer.



Figure S3. Time-dependent boron accumulation in HeLa cells. The cells $(1 \times 10^6 \text{ cells})$ were incubated at 37 °C for 3 h in a medium containing boron compounds (100 ppm [B]). After the incubation, the cells were washed three times with PBS and digested with 2 mL of perchloric acid/hydrogen peroxide at 70 °C 1 h. Boron concentrations in the solution were determined by an inductively coupled plasma optical emission spectroscopy (ICP-OES).



Figure S4. The concentration-dependent cell viability of folate in HeLa cells. Significant antiproliferative effect was not observed up to a 12 mM concentration of folate.