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Preparation of Porphyrins Having Phenylboronic Acid Groups

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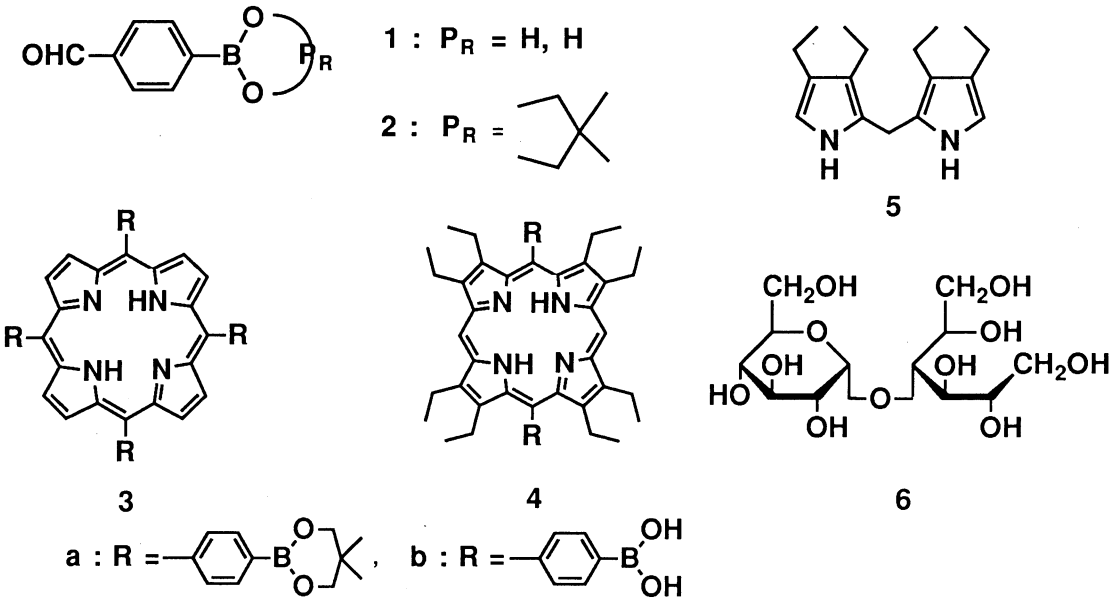
Tetra- and disubstituted porphyrins with *p*-dihydroxyborylphenyl groups on their meso-positions have been prepared as BNCT target compounds. The resulting porphyrins were solubilized upon formation of sugar-boronic acid complexes.

BNCT (Boron neutron capture therapy) proposed by Locher in 1936 has been of interest as a very promissing anti-cancer treatment.[1] It requires a compound as boron carrier with high affinity for tumor cells so as to transport considerable amounts of B-10 atoms only into the targeted malignant tissue without accumulation in the surrounding normal tissues. Although a number of boron containing compounds or antibody conjugates have been prepared for BNCT,[2-4] higher selectivity for tumor to normal tissue is still required.

Porphyrins have been practically utilized as tumor labeling agents for diagnosis and as photosensitizers for photodynamic therapy, another type of anti-cancer treatment.[5] Porphyrins are shown to stay more than 24 h in tumor cells but less than several hours in normal cells after administration. Therefore, boronated porphyrins will be good tumor-targeted boron-carriers. Very recently, preparations of porphyrins bearing carborane groups for BNCT were reported.[4] We report here preparation of porphyrins having phenylboronic acid groups as boron function groups. The C-B bond in the phenylboronic acid group exhibits good stability under aerobic and aqueous conditions; the stability is one of the essential requirements in using boron compounds in BNCT. Furthermore, the boronic acid residue is also expected to enhance the solubility of the compound in water.

The boron containing porphyrin **3** was prepared from pyrrole and an equivalent amount of *p*-dihydroxyborylbenzaldehyde (**1**). Boronated benzaldehyde **1** was prepared by Sommelet reaction of *p*-tolylboronic acid in 35% yield.[6] 2,2-Dimethylpropane-1,3-diol was used to protect the boronic acid residue of **1** to avoid undesired reaction on the electrophilic boron atom. The protected aldehyde **2** (1.8 mmol) was allowed to react with an equivalent amount of pyrrole in $CHCl_3$ (50 mL) at room temperature for 24 h under N_2 atmosphere in the presense of *p*-toluenesulfonic acid (0.23 mmol) as catalyst. Oxidation by *p*-chloranil (1.6 mmol) followed by purification by means of column chromatography (silica gel / $CHCl_3$:EtOAc=1:1) and crystallization from $CHCl_3$-hexane afforded the porphyrin **3a** (-3% after purification). Optimization of the reaction conditions according to the suggestion of Lindsey *et al.* (0.01 M (1 M=1 mol dm^{-3}) for the reactants and 0.003 M for BF_3-OEt_2 as catalyst)[7] improved the yield of **3a** up to ca. 30%. Thus obtained porphyrin exhibits a typical etio type electronic absorption spectrum ; λmax in nm (log ε) 420(5.50), 517(4.16), 551(3.92), 591(3.74), and 648(3.57). The ^1H NMR signals (δ 8.81 (s, 8H, pyrrole-H), 8.20 and 8.15 (8H for each, d, J=8.0 Hz, phenyl-H), 3.93 (16H, s, -CH_2-), 1.15 (24H, s, -CH_3), and -2.80 (2H, br s, NH)) and elemental analysis ($C_{64}H_{66}N_4O_8B_4$; C,H,N) were in agreement with the structure of **3a**.



Octaethylporphyrin derivative **4a** was also synthesized readily. Tetraethyldipyrromethane **5** is known to cyclize readily with aromatic aldehydes affording porphyrin macrocycles.[8] Reaction of **2** (1.6 mmol) with an equivalent amount of **5** in methanol (15 mL) in the presense of *p*-toluenesulfonic acid (0.6 mmol) at 0 °C for 36 h under N_2 atmosphere afforded precipitates of porphyrinogen. Oxidation of the porphyrinogen with *p*-chloranil (1.6 mmol) in THF (10 mL) gave porphyrin **4a** in 44% yield after purification by column chromatography (silica gel / $CHCl_3$:EtOAc=1:1) and crystallization from $CHCl_3$-hexane. Protection of

boronic acid residue with N-methyl-bis(ethanol)amine did not give a sufficient result: only trace of porphyrin product was obtained under otherwise identical reaction conditions. Electronic absorption spectrum of **4a** (λmax in nm (log ε): 413(5.22), 510(4.06), 544(3.62), 577(3.77), 628(3.00)) is quite similar to those of 5,15-diaryl-octaethylporphyrins,[8] and the ^{1}H NMR spectrum (δ 10.21 (2H, s, meso-H), 8.16, 8.09 (4H, for each, d, J=7.7 Hz, phenyl-H), 4.00-3.96 (16H, m, -C$\underline{H}_2$-CH_3 and -C$\underline{H}_2$-), 2.78 (8H, q, J=7.3 Hz, -C$\underline{H}_2CH_3$), 1.84 (12H, t, J=7.3 Hz, -C$\underline{H}_2CH_3$), 1.19-1.13 (24H, m, -CH_2C$\underline{H}_3$ and -C$\underline{H}_3$)) confirms the structure of **4a**.

^{11}B NMR spectra of **3a** and **4a** proved the existense of boron atoms. Signals were observed with reasonable intensities at similar magnetic fields where alkyl esters of phenylboronic acid resonate. However, the linewidths were unusually broad (-2200 Hz), as compared with a linewidth of -230Hz for phenylboronic acid or its esters. Similar unusual line broadening has also been reported for a 3-biphenylyldiethylborane ,[9] where electoronic symmetry around the boron atom seems quite low. Such line broadening of ^{11}B NMR has been attributed mainly to quadrupole relaxation of ^{11}B atom.

Removal of the protecting group was achieved by succesive hydrolysis of porphyrin **3a** and **4a** in refluxing alkalline ethanol. The resulting porphyrins, however, were scarcely soluble in water around pH 7, although **3b**, but not **4b**, could be dissolved in water at pH 12. To obtain higher solubilty of these porphyrins in neutral water, we examined complexation with sugars at the boronic acid residue. Boric acid and Boronic acids are known to undergo reversible boronic ester formation with 1,2- or 1,3-diol groups readily even in aqueous media. Among sugars, maltitol (**6**), which comprises acyclic sugar alcohol (sorbitol) and glucopyranose, showed an excellent ability of complexation with these porphyrin-boronic acids so as to dissolve them in water. Porphyrin **3b** in chloroform (0.26 mM, 5mL, containing 4% acetone for complete dissolution) was extracted into water at pH 7.0 containing maltitol (0.3 M, 5 mL) in -80% yield, and **4b** was also extracted at pH 12 in a similar yield. Monosaccharides such as glucose and fructose were far less effective, and sorbitol afforded an emulsion layer. As the apparent association constants of fructose and solbitol with phenylboronic acid in water are almost the same, complexation on the boronic acid residue must not be the sole factor. In maltitol, the binding site to the boronic acid should be the sorbitol moiety and the glucose moiety may enhance water solubility. Ono *et al.* have recently reported water soluble porphyrins with covalently bonded sugar groups, and they have also expected one of those to have different affinity to cancer cells from known porphyrin agents.[10] The present study suggests that the phenylboronic acid residue is promising not only as a neutron capturing group but also as a solubility-enhancing group with the aid of polyols which are expected to interact selectively with cell membrane.

A preliminaly *in vivo* examination on the toxicity of the compound was carried out. The hydrolyzed porphyrin **3b** as a phosphate buffer-saline suspension containing 1% Tween-80 was injected in tumor-bearing mice, and no apparent severe toxicity was observed at a dose of 20 mg/kg. Further works involved in the tumor affinity of the compounds and complexation with sugars are now in progress.

References

1) H. Hatanaka, "Boron-Neutron Capture Therapy for Tumors," ed by H. Hatanaka, Nishimura Co., Niigata (1986), pp. 1.

2) R. G. Fairchild, "Proc. 2nd Int. Symp. on Neutron Capture Therapy," ed by H. Hatanaka, Nishimura Co., Niigata (1986), pp. 394; F. Alam, A. H. Soloway, B. V. Bapat, R. F. Barth, and D. M. Adams, *Basic Life Science*, **1989**, 107.

3) For recent papers, see: H. Ketz, W. Tjarks, and D. Gabel, *Tetrahedron Lett.*, **31**, 4003 (1990); K. Nagasawa and M. Narisada, *ibid.*, **31**, 4029 (1990); M. F. Hawthorne, A. Varadarajan, C. B. Knobler, S. Chakrabatri, R. J. Paxton, B. G. Beatty, and F. L. Curtis, *J. Am. Chem. Soc.*, **112**, 5365 (1990); Y. Yamamoto, T. Seko, F. Rong, and H. Nemoto, *Tetrahedron Lett.*, **30**, 7191 (1989); Y. Yamamoto, T. Seko, and H. Nemoto, *J. Org. Chem.*, **54**, 4734 (1989); H. Nemoto, F. Rong, and Y. Yamamoto, *ibid.*, **55**, 6055 (1990); J. L. Mauer, F. Berchier, A. J. Serino, C. B. Knobler, and M. F. Hawthorne, *ibid.*, **55**, 838 (1990).

4) M. Miura, D. Gabel, G. Obenbrink, and R. G. Fairchild, *Tetrahedron Lett.*, **31**, 2247 (1990); S. B. Kahl and M-S. Koo, *J. Chem. Soc., Chem. Commun.*, **1990**, 1769.

5) For example, H. Kato, K. Aizawa, H. Shinohara, C. Konaka, K. Nishimiya, N. Kawate, K. Yoneyama, H. Takahashi, M. Saito, H. M. Shyan, T. Tomono, K. Kinoshita, I. Yoshihama, and Y. Hayata, *Lasers in the Life Sciences*, **1**, 13 (1986), and references cited in.

6) H. R. Snyder, A. J. Reedy, and Wm. J. Lennarz, *J. Am. Chem. Soc.*, **80**, 835 (1958).

7) J. S. Lindsey, H. C. Hsu, and I. C. Schreiman, *Tetrahedron Lett.*, **27**, 4969 (1986); R.W.Wagner, D. S. Lawrence, and J. S. Lindsey, *ibid.*, **28**, 3069 (1987).

8) H. Ogoshi, H. Sugimoto, T. Nishiguchi, Y. Matsuda, and Z. Yoshida, *Chem. Lett.*, **1978**, 29; T. Young and C. K. Chang, *J. Am. Chem. Soc.*, **107**, 898 (1985).

9) R. Koester and H. G. Willemsen, *Liebigs Ann. Chem.*, **1974**, 1843.

10) N. Ono, M. Bougauchi, and K. Maruyama, *Tetrahedron Lett.*, **33**, 1629(1992).

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