BORONATED CONJUGATES OF PROTOHEMIN IX WITH L-AMINO ACIDS: SYNTHESIS AND ANTITUMOR ACTIVITY

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We report the synthesis of novel conjugates of protohemin IX with neutral and anionic boron polyhedra and L-amino acids. The amino acids are linked to the porphyrin macrocycle via the amide or ester bond. The serine containing boronated protohemin was the most cytotoxic for K562 human leukemia cell line. This compound interacted with double-stranded DNA in vitro and caused apoptosis of tumor cells including those that are resistant to several chemotherapeutic drugs.

Keywords: Protohemin IX; L-Amino acids; Cytotoxicity; DNA fragmentation; Antitumor drugs.

Porphyrins accumulate predominantly in tumor cells. This valuable feature makes natural and synthetic porphyrins attractive for conjugation with boron compounds and useful for boron neutron capture therapy of cancer (BNTC)[1,2]. Boronated porphyrins have been recently proposed to be employed for delivery of boron to tumors in BNTC and as photosensitizers in photodynamic therapy (PDT)[3,4]. Furthermore, boronated porphyrins can be visualized in cells and tissues by fluorescent microscopy. In the cells boronated porphyrins can interact with DNA due to their planar structure[5,6]. These characteristics together with physical treatments (thermal

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neutrons or monochromatic light) allow for triggering DNA damage and tumor cell death.

In spite of high therapeutic efficacy of individual carboranylporphyrins, their side effects remain a problem. For example, thrombosis and erythropenia might emerge even before the therapeutically sufficient concentration of boron in tumor is reached[7].

Water solubility, low dark toxicity, stability in the body and preferential concentration in the tumor compared with non-malignant tissues are the critical requirements for clinically applicable boronated porphyrins. We hypothesized that these requirements can be fulfilled using chemical modifications, namely, conjugation of natural protohemin IX with neutral and anionic carboranes and amino acids at the periphery of the porphyrin macrocycle. In particular, the amino acid residues are assumed to increase the amphiphilicity and to increase the accumulation of the conjugates in proliferating cells. Literature data suggest that the amino acid residues increase the transmembrane transport of the conjugates and stabilize the interaction of boronated porphyrins with DNA[8].

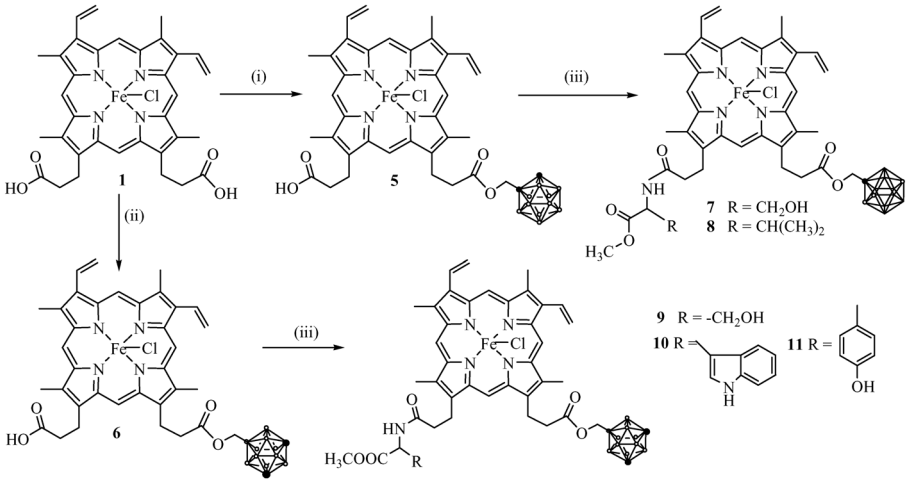
RESULTS AND DISCUSSION

Protohemin IX (**1**), a major component of hemoglobin, was used for synthesis of boronated derivatives. The following carborane polyhedra were utilized: 1-(hydroxymethyl)-*o*-carborane (**2**), 9-(hydroxymethyl)-*m*-carborane (**3**) and 1-(hydroxymethyl)-*closo*-monocarbon carboranyl cesium (**4**) in which the functional hydroxymethyl group is bonded to the carbon atom (**2**, **4**) or to the boron atom (**3**). As the L-amino acid components, methyl esters of serine, valine, tyrosine, phenylalanine and tryptophan as well as oxazaborolidine complexes of serine and threonine were used.

The strategy of synthesis of amino acid–carboranylporphyrin conjugates included the sequential addition of carborane to porphyrin and then the amino acid to carboranylporphyrin. The major problem was to obtain the boronated derivatives of porphyrin **1** substituted at only one carboxylic group. Previously we reported the methods of synthesis of natural type disubstituted carboranylporphyrins[9,10]. In the present study monosubstituted carborane derivatives **5** and **6** of porphyrin **1** were prepared using mixed-anhydride activation of carboxylic groups in porphyrin **1** with pivaloyl chloride (PivCl) in pyridine (compound **5**) or di-*tert*-butyl dicarbonate (Boc_2O) (compound **6**) followed by the treatment with carboranes **2** and **3**, respectively (Scheme 1). Carboranylporphyrins **5** and **6** in which carborane polyhedra are bonded to the porphyrin via the ester bond were prepared in

50–60% yield. For activation, PivCl was used in a two-fold excess whereas Boc_2O was used in the equimolar ratio.

The conjugation of the carboxylic group of porphyrin **5** or **6** with methyl esters of aliphatic and aromatic amino acids was performed with the use of Boc_2O in pyridine to afford serine- (**7**, **9**), valine- (**8**), tryptophan- (**10**) and tyrosine- (**11**) substituted derivatives in 40–80% yields.



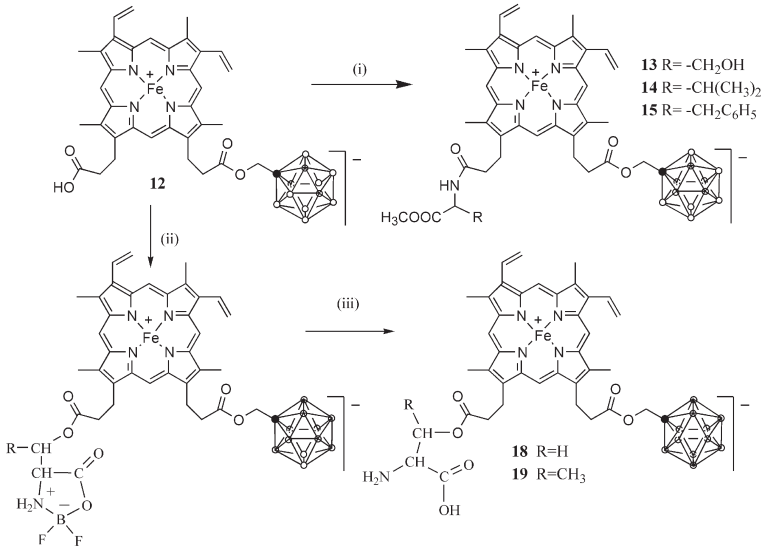
(i) 1. PivCl-Et_3N, Py-CH_2Cl_2 ,2. **2**, DMAP; (ii) 1. $(Boc)_2O$, Py-CH_2Cl_2 , 2. **3**, DMAP
(iii) 1. $(Boc)_2O$, Py-CH_2Cl_2 , 2. L-amino acid methyl ester, DMAP

SCHEME 1

We also obtained amino acid conjugates of boronated protohemin[11] (**12**) containing anionic *closo*-monocarbon carborane as substituent. At concentrations that caused no dark toxicity, **12** was highly efficient in photodynamic treatment of transplanted rat M-1 sarcoma[11].

We obtained two types of amino acid derivatives of porphyrin **12**. In the first type the L-amino acids were linked to porphyrin **12** via the amide bond, and in the second type via the ester bond. The amide derivatives were obtained by the reactions of methyl esters of L-serine, L-valine or L-phenylalanine with Boc-activated carboxylic group of porphyrin **12** in pyridine–CH_2Cl_2 system (Scheme 2).

The reaction of **12** with oxazaborolidine complexes[12] of L-serine (**16**) or L-threonine (**17**) followed by hydrolysis of intermediates yielded the amino acid conjugates **18** and **19** in which the amino acid residue was linked to porphyrin **12** via ester bond (Scheme 2). Importantly, compounds **18** and **19** possess free amino and carboxy groups that allow for higher water solubility of the conjugates.



(i) 1. Boc_2O, Py-CH_2Cl_2 , 2. L-amino acid methyl ester, DMAP; (ii) 1. Boc_2O, Py-CH_2Cl_2 , 2. **16** or **17**, DMAP; (iii) H_2O

SCHEME 2

The synthesized compounds were purified by column chromatography using the system $CHCl_3$–MeOH 9:1 as eluent. Porphyrins **7**–**11**, **13**–**15**, **18** and **19** were dark-red crystals soluble in CH_2Cl_2, $CHCl_3$, MeOH and THF. The structures of novel compounds were confirmed by IR, electron spectroscopy and mass spectrometry.

The difference in UV-VIS spectra of **18** and **19** is noteworthy. It reflects the essential changing in electron density distribution caused by substitution of hydrogen atom of the amino acid moiety in porphyrin **18** with electron-donating methyl group in porphyrin **19**.

We compared the cytotoxicity of novel amino acid containing porphyrins with anionic monocarboncarborane (**13**–**15**) for cultured K562 human leukemia cell line. Cells were treated with up to 50 μM of each compound for 72 h followed by colorimetric MTT-test[13]. Serine-containing compound **13** was the most cytotoxic (IC_{50} ~ 5 μM). In contrast, structurally close valine-containing compound **14** was substantially less potent (IC_{50} ~ 35 μM) whereas carboranylporphyrin with aromatic amino acid **15** was inert. Compound **12** was non-toxic. These results indicate that the introduction of the serine residue makes the entire complexes cytotoxic. High antitumor potency of serine-containing carboranylporphyrin was also demonstrated for MCF-7 (breast carcinoma), CaOv (ovarian carcinoma) and HCT116 (colon

carcinoma) human cell lines. The reasons for preferential cytotoxicity of **13** are under investigation; one may hypothesize that this fact is due to higher amphiphilicity of this derivative.

Because the serine-containing monocarbon carboranylporphyrin **13** showed the highest cytotoxicity, we next studied the mechanisms that might contribute to this important antitumor characteristic. We performed spectrophotometric analysis of saline solutions of native thymus DNA [14] in the presence of serine-containing compounds. These interactions were detectable as a shift of maximum DNA absorbance from 260 to 273 nm. We found that **13** interacted with double-stranded DNA (Fig. 1). Furthermore, compound **18** in which serine is conjugated with the porphyrin macrocycle via ester bond, also showed a DNA binding activity (Fig. 1). In contrast, protohemin IX (**1**) and boronated protohemin (**5**) did not bind DNA (not shown). Thus, conjugation of serine residue conferred novel feature for boronated porphyrins, namely, the ability to bind the duplex DNA.

Since **13** serine-containing monocarbon we tried to determine whether carboranylporphyrin caused DNA fragmentation, a hallmark of apoptosis. The DNA content in K562 leukemia cells treated with **13** was measured by flow cytometry. The percentage of degraded DNA depended on the concentration of **13** and time of treatment. After 24 h of cell exposure to 10 μM **13**, >40% of DNA was hypodiploid, and after 48 h it was >60% (Fig. 2). These results indicated that serine-containing carboranylporphyrin induced massive DNA degradation and apoptosis.

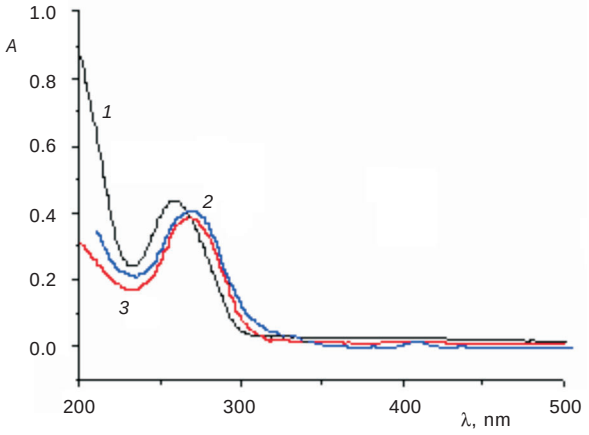


FIG. 1
UV-VIS spectra of aqueous solutions: *1* DNA, *2* DNA incubated with **13**, *3* DNA incubated with **18**

Finally, given that **13** demonstrated the highest cytotoxicity, we selected this compound for the capability to circumvent drug resistance in tumor cells. Cell lines with major determinants of anticancer drug resistance, i.e., expression of transmembrane P-glycoprotein and deletion of pro-apoptotic p53. We found that our novel amino acid derivatives of boronated porphyrin were not transported by P-glycoprotein. Furthermore, serine-containing carboranylporphyrin caused death of HCT116 cells (carrying wild type p53) and their isogenic $p53^{-/-}$ subline within a similar range of concentrations.

In summary, we synthesized a series of boronated porphyrins conjugated with L-amino acid residues. The novel water-soluble compounds showed differential cytotoxicity for cultured human leukemia cells. Conjugation of porphyrins with monocarbon carborane and serine yielded the compounds perspective for further investigation as anticancer chemotherapeutic agents. Our findings broaden the applicability of boronated porphyrins beyond their conventional use as photo/radiosensitizers.

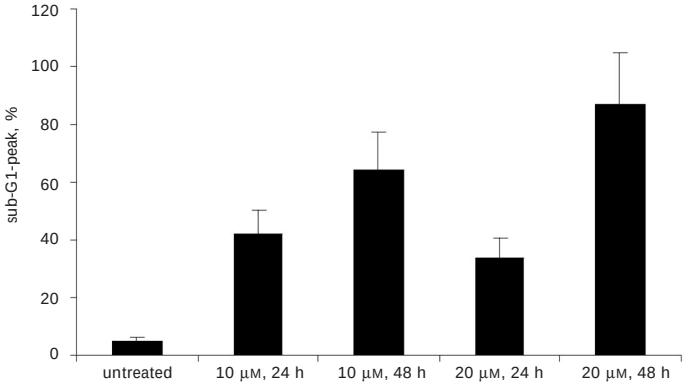


FIG. 2
DNA fragmentation induced by serine-containing carboranylporphyrin **13** in K562 cells

EXPERIMENTAL

IR spectra (wavenumbers in cm^{-1}) were recorded on a Specord M-82 (Carl Zeiss) in KBr. The UV-VIS spectra (wavelengths in nm) were measured on a spectrophotometer Jasco UV 7800 in $CHCl_3$. MS data were determined by MALDI on a Vision 2000 mass spectrometer. Carboranes **2** [15], **3** [16], **4** [17] and oxazaborolidine complexes[12] of L-serine **16** and L-threonine **17** were prepared previously. Silica gel Merk L (0.040-0.08) was used for column chromatography (eluent $CHCl_3$–MeOH 9:1). Purity of compounds was checked by TLC on Silufol plates with a $CHCl_3$–MeOH 9:1 solvent system. The solvents were purified according to standard

procedures. Cell culture, drug treatment and cytotoxicity assays were performed as described[11]. Cytotoxicity of novel compounds was tested in MTT-test as described[14].

1,3,5,8-Tetramethyl-2,4-divinyl-6(7)-[2′-(*o*-carboran-1′-yl)methoxycarbonylethyl]-7(6)-(2′-carboxyethyl)porphyrin Iron(III) Chloride (**5**)

Triethylamine (35 mg, 0.35 mmol) was added to a solution of porphyrin **1** (100 mg, 0.15 mmol) in a mixture of CH_2Cl_2 (8 ml) and Py (8 ml) at 0 °C. The reaction mixture was cooled to –15 °C, a solution of PivCl (37 mg, 0.35 mmol) in CH_2Cl_2 (3 ml) was added dropwise over 0.5 h, and the mixture was kept at this temperature for 0.5 h. A solution of carborane **2** (52 mg, 0.3 mmol) in CH_2Cl_2 (3 ml) was added at 0 °C and the mixture was stirred at 20 °C for 1.5 h. Then it was poured into 2% HCl (150 ml) and extracted with $CHCl_3$ (150 ml). The extract was washed with water (3 × 200 ml) to pH 7 and dried over anhydrous Na_2SO_4. After removal of $CHCl_3$ in vacuo, the residue was isolated by column chromatography on silica gel to give 62 mg (53%) of pure **5**. UV: 387 (Soret), 510, 539, 642 (1:0.10:0.09:0.05). IR: 3080 (carborane CH), 2976, 2942 (CH), 2600 (BH), 1730 (CO, ester), 1715 (CO, COOH). For $C_{37}H_{44}B_{10}ClFeN_4O_4$ (808.2) calculated: 54.99% C, 5.49% H, 13.38% B, 6.93% N; found: 55.28% C, 5.36% H, 13.65% B, 6.97% N. MS (MALDI, *m/z*): 774 [M^+ – Cl].

1,3,5,8-Tetramethyl-2,4-divinyl-6(7)-[2′-(*o*-carboran-9″-yl)methoxycarbonylethyl]-7(6)-(2′-carboxyethyl)porphyrin Iron(III) Chloride (**6**)

Di-*tert*-butyl dicarbonate (67 mg, 0.3 mmol) was added to a solution of porphyrin **1** (200 mg, 0.3 mmol) in a mixture of CH_2Cl_2 (10 ml) and Py (10 ml) at 0 °C followed by stirring for 15 min to form the mixed anhydride (TLC in $CHCl_3$–MeOH 9:1, R_F 0.6). Then a solution of carborane **3** (50 mg, 0.3 mmol) in CH_2Cl_2 (5 ml) and DMAP (20 mg) were added and the mixture was kept at 20 °C for 3 h. After removal of the solvents in vacuo, the residue was isolated by column chromatography on silica gel to give 144 mg (62%) of pure **8**. UV: 387.8 (Soret), 511, 542, 643 (1:0.1:0.1:0.05). IR: 3059 (carborane CH), 2917, 2850 (CH), 2585 (BH), 1725 (CO, ester), 1716 (CO, COOH). For $C_{37}H_{44}B_{10}ClFeN_4O_4$ (808.2) calculated: 54.99% C, 5.49% H, 13.38% B, 6.90% N; found: 55.479% C, 5.39% H, 13.71% B, 7.05% N. MS (MALDI, *m/z*): 773 [M^+ – Cl].

Synthesis of L-Amino Acid Amide Conjugates **7**–**11** and **13**–**15**. General Procedure

Di-*tert*-butyl dicarbonate (22 mg, 0.1 mmol) was added to a solution of carboranylporphyrin (**5**, **6** or **12**) (0.06 mmol) in a mixture of CH_2Cl_2 (6 ml) and Py (6 ml) at 0 °C followed by stirring for 15 min. Then corresponding L-amino acid methyl ester (0.1 mmol) in CH_2Cl_2 (3 ml) and DMAP (10 mg) were added and the mixture was stirred at 20 °C for 3 h. After removal of the solvents in vacuo, the residue was purified by column chromatography on silica gel.

1,3,5,8-Tetramethyl-2,4-divinyl-6(7)-[2′-(o-carboran-1″-yl)methoxycarbonylethyl]-7(6)-[2′-N^{α}(O^{α}-methyl-L-seryl)carbonylethyl]porphyrin iron(III) chloride (**7**). From L-serine methyl ester (12 mg, 0.1 mmol) and porphyrin **5** (46 mg, 0.06 mmol), 16 mg (37%) of compound **7** were obtained. UV: 387 (Soret), 510, 539, 642 (1:0.12:0.11:0.05). IR: 3354 (OH), 3057 (carborane CH), 2922, 2850 (CH), 2577 (BH), 1739, 1732 (CO, ester), 1660 (amide I), 1550 (amide II). For $C_{41}H_{51}B_{10}ClFeN_5O_6$ (909.3) calculated: 54.16% C, 5.65% H, 11.89% B, 6.14% N; found: 54.85% C, 5.53% H, 12.32% B, 6.09% N. MS (MALDI, *m/z*): 841 [M^+ – Cl].

1,3,5,8-Tetramethyl-2,4-divinyl-6(7)-[2′-(o-carboran-1″-yl)methoxycarbonylethyl]-7(6)-[2′-N$^{\alpha}$(O$^{\alpha}$-methyl-L-valyl)carbonylethyl]porphyrin iron(III) chloride (**8**). From L-valine methyl ester (13 mg, 0.1 mmol) and porphyrin **5** (46 mg, 0.06 mmol), 48 mg (84%) of compound **8** were obtained. UV: 387 (Soret), 507, 540, 640 (1:0.14:0.12:0.06). IR: 3060 (carborane CH), 2917, 2849 (CH), 2599 (BH), 1738 (CO, ester), 1645 (amide I), 1558 (amide II). For $C_{44}H_{57}B_{10}ClFeN_5O_5$ (935.4) calculated: 56.50% C, 6.14% H, 11.56% B, 5.97% N; found: 56.14% C, 6.27% H, 12.00% B, 5.83 N. MS (MALDI, *m/z*): 899 [M^+ – Cl].

1,3,5,8-Tetramethyl-2,4-divinyl-6(7)-[2′-(o-carboran-9-yl)methoxycarbonylethyl]-7(6)-[2′-N$^{\alpha}$(O$^{\alpha}$-methyl-L-seryl)carbonylethyl]porphyrin iron(III) chloride (**9**). From L-serine methyl ester (12 mg, 0.1 mmol) and porphyrin **6** (46 mg, 0.06 mmol), 31 mg (55%) of compound **9** were obtained. UV: 388 (84.15), 508 (9.57), 540 (8.04), 641 (3.4) (1:0.11:0.10:0.04). IR: 3343 (NH), 2997, 2920 (CH), 2596 (BH), 1736 (CO, ester), 1623 (amide I), 1586 (amide II), 1462 (-CH=CH_2), 989 (-CH=CH_2). For $C_{41}H_{51}B_{10}ClFeN_5O_6$ (909.3) calculated: 54.16% C, 5.65% H, 11.89% B, 7.70% N; found: 54.24% C, 5.57% H, 11.83% B, 7.75% N. MS (MALDI, *m/z*): 874 [M^+ – Cl].

1,3,5,8-Tetramethyl-2,4-divinyl-6(7)-[2′-(o-carboran-9″-yl)methoxycarbonylethyl]-7(6)-[2′-N$^{\alpha}$(O$^{\alpha}$-methyl-L-tryptophyl)carbonylethyl]porphyrin iron (III) chloride (**10**). From L-tryptophan methyl ester (22 mg, 0.1 mmol) and porphyrin **6** (46 mg, 0.06 mmol), 27 mg (72%) of compound **10** were obtained. UV: 387 (Soret), 511, 540, 645 (1:0.1:0.10:0.05). IR: 3056 (carborane CH), 2920 (CH), 2604 (BH), 1740 (CO, ester), 1645 (amide I), 1515 (amide II). For $C_{49}H_{56}B_{10}ClFeN_6O_5$ (1008.4) calculated: 58.36% C, 5.60% H, 10.72% B, 5.54% N; found: 57.98% C, 5.54% H, 11.5% B, 5.41% N. MS (MALDI, *m/z*): 972 [M^+ – Cl].

1,3,5,8-Tetramethyl-2,4-divinyl-6(7)-[2′-(o-carboran-9″-yl)methoxycarbonylethyl]-7(6)-[2′-N$^{\alpha}$(O$^{\alpha}$-methyl-L-tyrosyl)carbonylethyl]porphyrin iron(III) chloride (**11**). From L-tyrosine methyl ester (19 mg, 0.1 mmol) and porphyrin **6** (46 mg, 0.06 mmol), 60 mg (79%) of compound **11** were obtained. UV: 397 (Soret), 495, 534, 609 (1:0.11:0.09:0.06). IR: 3362 (OH), 3065 (carborane CH), 2923, 2851 (CH), 2601 (BH), 1740 (CO, ester), 1660 (amide I), 1560 (amide II). For $C_{47}H_{55}B_{10}ClFeN_5O_6$ (985.4) calculated: 57.29% C, 5.63% H, 10.97% B, 7.11% N; found: 56.92% C, 5.79% H, 11.36% B, 7.21% N. MS (MALDI, *m/z*): 950 [M^+ – Cl].

1,3,5,8-Tetramethyl-2,4-divinyl-6(7)-[2′-(closo-monocarbon-carboran-1"-yl)methoxycarbonylethyl]-7(6)-[2-N$^{\alpha}$(O$^{\alpha}$-methyl-L-seryl)carbonylethyl]porphyrin iron(III) (**13**). From L-serine methyl ester (12 mg, 0.1 mmol) and porphyrin **12** (46 mg, 0.06 mmol), 13 mg (46%) of compound **13** were obtained. UV: 398 (Soret), 511, 542, 644 (1:0.11:0.10:0.04). IR: 3338 (NH, OH), 2947 (CH), 2540 (BH), 1742 (CO, ester), 1658 (amide I), 1553 (amide II). For $C_{40}H_{51}B_{11}FeN_5O_6$ (872.6) calculated: 55.06% C, 5.89% H, 13.63% B, 8.03% N; found: 55.21% C, 5.72% H, 13.64% B, 8.16% N. MS (MALDI, *m/z*): 872 [M^+].

1,3,5,8-Tetramethyl-2,4-divinyl-6(7)-[2′-(closo-monocarbon-carboran-1"-yl)methoxycarbonylethyl]-7(6)-[2-N$^{\alpha}$(O$^{\alpha}$-methyl-L-valyl)carbonylethyl]porphyrin iron(III) (**14**). From methyl ester of L-valine hydrochloride (13 mg, 0.1 mmol) and porphyrin **12** (46 mg, 0.06 mmol), 18 mg (59%) of compound **14** were obtained. UV: 386 (Soret), 511, 542, 643 (1:0.11:0.10:0.05). IR: 3383 (NH), 2970 (CH), 2545 (BH), 1719 (CO, ester), 1657 (amide I), 1623 (C=C), 1554 (amide II). For $C_{42}H_{55}B_{11}FeN_5O_5$ (884.7) calculated: 57.02% C, 6.27% H, 13.44% B, 7.92% N; found: 56.77% C, 6.35% H, 13.87% B, 7.83% N. MS (MALDI, *m/z*): 884 [M^+].

1,3,5,8-Tetramethyl-2,4-divinyl-6(7)-[2′-(closo-monocarbon-carboran-1"-yl)methoxycarbonylethyl]-7(6)-[2-N$^{\alpha}$(O$^{\alpha}$-methyl-L-phenylalanyl)carbonylethyl]porphyrin iron(III) (**15**). From L-phenylalanine methyl ester (18 mg, 0.1 mmol) and porphyrin **12** (46 mg, 0.06 mmol), 18 mg (59%) of compound **15** were obtained. UV: 396 (Soret), 511, 542, 643 (1:0.13:0.10:0.06). IR: 3389

(NH), 2930 (CH), 2540 (BH), 1725 (CO, ester), 1674 (amide I), 1625 (C=C), 1550 (amide II). For $C_{46}H_{55}B_{11}FeN_5O_5$ (932.7) calculated: 59.23% C, 5.94% H, 12.75% B, 7.51% N; found: 59.13% C, 5.87% H, 12.89% B, 7.46% N. MS (MALDI, *m/z*): 932 [M^+].

Synthesis of L-Amino Acid Ester Conjugates **18** and **19**. General Procedure

Di-*tert*-butyl dicarbonate (22 mg, 0.1 mmol) was added to a solution of porphyrin **12** (0.07 mmol) in CH_2Cl_2 (4 ml) and Py (4 ml) at 0 °C followed by stirring for 15 min. Then a solution of the oxazaborolidine complex of serine (**16**) or threonin (**17**) (0.3 mmol) in CH_2Cl_2 (3 ml) and DMAP (10 mg) were added, and the mixture was stirred at 20 °C for 2 h. Then H_2O (5 ml) was added to the reaction mixture and stirring was continued for 1 h for serine and 10 h for threonin. After removal of the solvents from the organic layer in vacuo, the residue was purified by column chromatography on silica gel.

1,3,5,8-Tetramethyl-2,4-divinyl-6(7)-[2′-(closo-monocarbon-carboran-1"-yl)methoxycarbonylethyl]-7(6)-[2-(O^{β}-L-seryl)carbonylethyl]porphyrin iron(III) (**18**). From oxazaborolidine complex **16** (46 mg, 0.3 mmol) and porphyrin **12** (50 mg, 0.07 mmol), 34 mg (60%) of compound **18** were obtained. UV: 363 (Soret), 511, 542, 621 (1:0.10:0.07:0.02). IR: 3700–3100 (OH), 3315 (NH), 2936 (CH), 2540 (BH), 1725 (CO, ester), 1623 (C=C). For $C_{39}H_{49}B_{11}FeN_5O_6$ (858.6) calculated: 54.56% C, 5.75% H, 13.85% B, 8.16% N; found: 54.63% C, 5.65% H, 13.91% B, 8.13% N. MS (MALDI, *m/z*): 858 [M^+].

1,3,5,8-Tetramethyl-2,4-divinyl-6(7)-[2′-(closo-monocarbon-carboran-1"-yl)methoxycarbonylethyl]-7(6)-[2-(O^{β}-L-threonyl)carbonylethyl]porphyrin iron(III) (**19**). From oxazaborolidine complex **17** (50 mg, 0.3 mmol) and porphyrin **12** (50 mg, 0.07 mmol), 30 mg (53%) of compound **19** were obtained. UV: 406 (Soret), 538, 641 (1:0.13:0.04). IR: 3700–3100 (OH), 3321 (NH), 2925 (CH), 2540 (BH), 1730 (CO, ester), 1623 (C=C). For $C_{40}H_{51}B_{11}FeN_5O_6$ (872.6) calculated: 55.06% C, 5.89% H, 13.63% B, 8.03% N; found: 55.21% C, 5.79% H, 13.69% B, 7.86% N. MS (MALDI, *m/z*): 872 [M^+].

Spectrophotometric Analysis of Carboranylporphyrin–DNA Binding

Into 3 ml of phosphate buffered saline, pH 7.2 in a 1 cm quartz cuvette, 3 μl of calf thymus DNA (final concentration 10 μg/ml) were added. Spectra were analyzed on a M-40 spectrophotometer (Carl Zeiss) in the 200–900 nm range. New compounds were added to the cuvette (final concentration of each porphyrin was 10 μM), and the spectra were recorded using a solution of DNA alone as reference. Interaction of DNA and the porphyrin was detected by shift of maximum absorbance of DNA. No absorbance in this region was detectable in the solution of the porphyrin alone.

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