Synthesis of Netropsin and Distamycin Analogues Bearing *o*-Carborane and Their DNA Recognition

Yoshinori Yamamoto,*,† Jianping Cai,† Hiroyuki Nakamura,† Naoki Sadayori,† Naoki Asao,‡ and Hisao Nemoto‡

Department of Chemistry, Faculty of Science, Tohoku University, Sendai 980-77, Japan and Department of Applied Molecular Science, Institute for Molecular Science, Okazaki 444, Japan

Received February 1, 1995 (Revised Manuscript Received March 28, 1995®)

Netropsin and distamycin A analogues containing *o*-carborane framework, **4a–c** and **5a–c**, respectively, were synthesized to investigate DNA binding sequence of these molecules. Cascade type polyols were attached to the carboranes in certain cases; **4b** and **5b** had the diol unit and **4c** and **5c** possessed the tetraol unit, whereas **4a** and **5a** had no hydroxy group. MPE·Fe(II) footprinting on the 216 base pair Pvu I/ Bam HI restriction fragment from pBLUESCRIPT KS(+1−) (bp 2958) indicated that **4a** and **5a** bound only slightly to the DNA fragment, whereas **4b** and **5b** bearing two hydroxy groups bound to the A,T-rich base pairs. The compounds containing four hydroxy groups **4c** and **5c** bound most selectively to the DNA fragments. In general, the compounds **5** containing three pyrrole rings in their molecules bound to the DNA more selectively than the corresponding two pyrrole ring-bearing compounds **4**.

The application of the cytotoxic ^{10}B neutron-capture reaction [$^{10}B(n,\alpha)^7Li$] to the treatment of human tumors has received much attention in recent years. The interaction of boron-10 isotope and thermal neutron produces an α-particle and recoils a lithium-7 ion bearing approximately 2.4 MeV. The heavy, charged particles, ^4He and ^7Li, have ranges in tissue of only 9 and 5 μm, respectively. Thus ionizing radiation is deposited preferentially in and around the tumor. The destructive effect is, therefore, highly localized to boron loaded tissue. Boron neutron capture therapy (BNCT) is a binary therapy in which a substance labeled with ^{10}B preferentially accumulates in a tumor before the tumor area is irradiated by slow neutrons.[1-4] A key requirement of BNCT is the selective delivery of an adequate concentration of boron-10 to tumors (15–30 μg ^{10}B/g tumor).[5] Boronated analogues of compounds that are known to localize in various tumors have been the focus of compounds developed in this area.[6] Carborane-substituted nucleosides,[7] boronic acid derivatives of pyrimidine and purine nucleosides,[8] nucleotide analogs containing boranophosphate linking groups,[9] nucleosides modified by base complexation with cyanoboran,[10] 2-thiouracil derivatives as melanoma-selective boron delivery species,[11] phenothiazine charge-transfer complexes with existing melanine[12] boron-containing amino acids,[13] carborane-containing steroids and hormones,[14] carborane-substituted porphyrins and phthalocyanines,[15] and boron conjugated antibodies and peptides[6,16] have been prepared and most of those potentially promising carriers have been tested in vitro or in vivo. Sodium mercaptoundecahydrododecaborate ($Na_2B_{12}H_{11}SH$) and 4-dihydroxyborylphenylalanine have been used clinically for treatment of brain tumors[1] and skin cancer,[2] respectively.

The possibility of localizing boron in the DNA of target cells by complexing it with boron-containing ligand molecules has recently been investigated by Whittaker

† Tohoku University.

‡ Institute for Molecular Science.

® Abstract published in *Advance ACS Abstracts,* May 15, 1995.

(1) Hatanaka, H. *Boron Neutron Capture Therapy for Tumors;* Nishimura Co. Ltd.: Niigata, Japan, 1986.

(2) Mishima, Y.; Honda, C.; Ichihashi, M.; Obara, H.; Hiratsuka, J.; Fukuda, N.; Karashima, H.; Kobayashi, T.; Kanda, K.; Yoshino, K. *Lancet* **1989**, 388.

(3) Fairchild, R. G.; Slatkin, D. N.; Caderre, J. A.; Micca, P. L.; Laster, B. H.; Kahl, S. B.; Som, P.; Fand, I.; Wheeler, F. *Pigment Cell Res.* **1989**, *2*, 309.

(4) *Clinical Aspects of Neutron Capture Therapy*; Fairchild, R. G., Bond, V. P.; Woodhead, A. D., Eds.; Plenum: New York, 1988.

(5) Fairchild, R. G.; Bond, V. P. *Int. J. Radiat. Oncol. Biol. Phys.* **1985**, *11*, 831.

(6) For an excellent review, Hawthorne, M. F. *Angew. Chem. Int. Ed. Engl.* **1993**, 32, 950.

(7) (a) Anisuzzaman, A. K. M.; Alam, F.; Soloway, A. H. *Polyhedron* **1990**, *9*, 891. (b) Tjarks, W.; Anisuzzaman, A. K. M.; Liang, L.; Soloway, A. H.; Barth, R. F.; Perkins, D. J.; Adams, D. M. *J. Med. Chem.* **1992**, *35*, 1628. (c) Tjarks, W.; Anisuzzaman, A. K. M.; Soloway, A. H. *Nucleosides Nucleotides* **1992**, *11*, 1765. (d) Yamamoto, Y.; Seko, T.; Nakamura, H.; Nemoto, H.; Hojo, H.; Mukai, N.; Hashimoto, Y. *J. Chem. Soc., Chem. Commun.* **1992**, 157. (e) Pyrimidine derivative; Reynolds, R. C.; Trask, T. W.; Sedwick, W. D. *J. Org. Chem.* **1991**, *56*, 2391.

(8) (a) Liao, T. K.; Podrebarac, E. C.; Cheng, C. C. *J. Am. Chem. Soc.* **1964**, *86*, 1869. (b) Schinazi, R. F.; Prusoff, W. H. *Tetrahedron Lett.* **1978**, 4981; *J. Org. Chem.* **1985**, *50*, 841. (c) Yamamoto, Y.; Seko, T.; Nemoto, H. *J. Org. Chem.* **1989**, *54*, 4734. (d) Yamamoto, Y.; Seko, T.; Rong, F.; Nemoto, H. *Tetrahedron Lett.* **1989**, *30*, 7191. (e) Yamamoto, Y. *Pure Appl. Chem.* **1991**, *63*, 423.

(9) (a) Spielvogel, B. F.; Sood, A.; Shaw, B. R.; Hall, I. H. *Pure Appl. Chem.* **1991**, *63*, 415. (b) Sood, A.; Shaw, B. R.; Spielvogel, B. F. *J. Am. Chem. Soc.* **1990**, *112*, 9000. (c) Tomasz, J.; Shaw, B. R.; Porter, K.; Spielvogel, B. F.; Sood, A. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1373.

(10) Sood, A.; Spielvogel, B. F.; Shaw, B. R. *J. Am. Chem. Soc.* **1989**, *111*, 9234.

(11) (a) Tjarks, W.; Gabel, D. *J. Med. Chem.* **1991**, *34*, 315. (b) Ketz, H.; Tjarks, W.; Gabel, D. *Tetrahedron Lett.* **1990**, *31*, 4003. (c) Wilson, J. G. *Pigment Cell. Res.* **1987**, *2*, 297.

(12) Nakagawa, T.; Ando, K. *Chem. Pharm. Bull.* **1976**, *24*, 778.

(13) (a) 4-Closo and 4-nido derivatives of DL-4-carboranylphenylalanine: Prashar, J. K.; Moore, D. E.; Wilson, J. G.; Allen, B. J. *Abstr. 5th Int. Symp. Neutron Capture Ther. Cancer* **1992**, 2. (b) L-Carboranylalanine: Radel, P. A.; Kahl, S. B. *Abstr. 5th Int. Symp. Neutron Capture Ther. Cancer* **1992**, 5. Sjöberg, S.; Hawthorne, M. F.; Lindstrom, P.; Malmquist, J.; Carlsson, J.; Andersson, A. Pettersson, O. *Ibid.* **1992**, 2. (c) Spielvogel, B. F. *Mol. Struct. Energ.* **1988**, *5*, 329. (d) Carborane containing aziridines: Yamamoto, Y.; Nakamura, H. *J. Med. Chem.* **1993**, *36*, 2232.

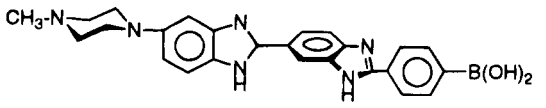
(14) (a) Wellmann, F.; Abraham, R.; Müller, R.; Gabel, D. *Z. Naturforsch. C* **1991**, *46*, 252. (b) Kahl, S. B. *Proc. First Int. Symp. Neutron Capture Ther.* **1983**, 294. (c) Hadd, H. E. *Ibid.* **1983**, p 281.

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

(16) (a) Kane, R. R.; Pak, R. H.; Hawthorne, M. F. *J. Org. Chem.* **1993**, *58*, 991. (b) Kane, R. R.; Lee, C. S.; Drechsel, K.; Hawthorne, M. F. *J. Org. Chem.* **1993**, *58*, 3227. (c) Kane, R. R.; Drechsel, K.; Hawthorne, M. F. *J. Am. Chem. Soc.* **1993**, *115*, 8853.

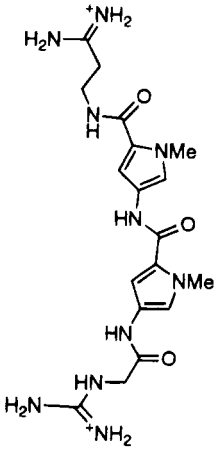
0022-3263/95/1960-3352$09.00/0

et al.[17a] and Corder et al.[17b] Dibenzimidazole reagents (for example, **1**) effectively bind to the minor groove of

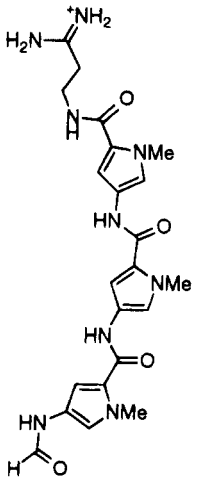


1

nuclear DNA, and **1** has been the subject of preliminary in vitro investigations of cellular boron uptake.[17] Netropsin **2** and distamycin A **3** are natural products that bind in the minor groove of double helical DNA at sites

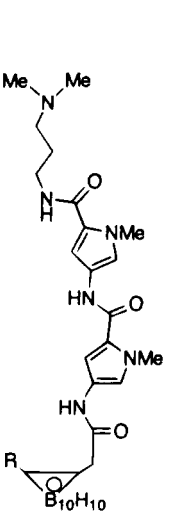


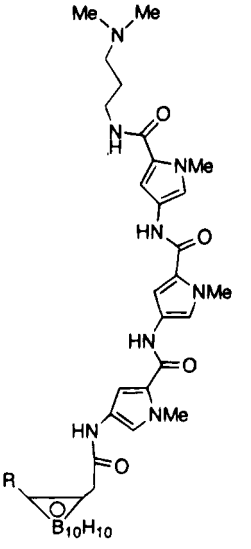
Netropsin (**2**)



Distamycin A (**3**)

of 4 or 5 successive A,T base pairs.[18-21] X-ray[22] and NMR[23] studies of netropsin-DNA and distamycin-DNA complexes reveal how sequence specificity is accomplished. It occurred to us that carborane attached netropsin and distamycin derivatives would bind to DNA, thereby sufficient amounts of boron atoms would be delivered to tumors.[24] Furthermore, footprinting experiments using MPE·Fe(II)[25] must tell us the binding sequences of **4** and **5** to DNA.





4a; R = H

b; R = CH_2O-(OH, OH)

c; R = CH_2O-(O-(OH, OH), O-(OH, OH))

5a; R = H

b; R = CH_2O-(OH, OH)

c; R = CH_2O-(O-(OH, OH), O-(OH, OH))

Results and Discussion

Synthesis of Carborane-Containing Netropsin and Distamycin Derivatives. 3-[1-Methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrole-2-carboxamido]-1-(dimethylamino)propane (**6**) and 3-{1-methyl-4-[1-methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido}-1-(dimethylamino)propane (**7**) were synthesized from *N*-methylpyrrole according to the Shibuya's procedure.[26] Synthe-

(17) (a) Whittaker, A. D.; Kelly, D. P.; Pardee, M.; Martin, R. F. *Proceedings of the 4th International Symposium on Neutron Capture Therapy for Cancer*, Allen, B. J., Moore, D. E., Harrington, B. V., Eds.; Plenum: New York, 1992; p 231. (b) Corder, A.; Martin, R. F.; Whittaker, A.; Kelly, D. P.; Meriaty, H.; Allen, B. J. *Abstr. 5th Int. Symp. Neutron Capture Ther. Cancer* **1992**, p 12.

(18) For reviews, see: Dervan, P. B. *Science* **1986**, *232*, 464. Zimmer, C.; Wähnert, U. *Prog. Biophys. Molec. Biol.* **1986**, *47*, 31.

(19) (a) Krylov, A. S.; Grokhovsky, S. L.; Zasedatelev, A. S.; Zhuze, A. L.; Gursky, G. V.; Gottikh, B. P. *Nucleic Acids Res.* **1979**, *6*, 289. (b) Zasedatelev, A. S.; Gursky, G. V.; Zimmer, Ch.; Thrum, H. *Mol. Biol. Rep.* **1974**, *1*, 337. (c) Zasedatelev, A. S.; Zhuze, A. L.; Zimmer, Ch.; Grokhovsky, S. L.; Tumanyan, V. G.; Gursky, G. V.; Gottikh, B. P. *Dokl. Akad. Nauk SSSR* **1976**, *231*, 1006.

(20) (a) Van Dyke, M. W.; Hertzberg, R. P.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 5470. (b) Van Dyke, M. W.; Dervan, P. B. *Cold Spring Harbour Symp. Quant. Biol.* **1982**, *47*, 347. (c) Van Dyke, M. W.; Dervan, P. B. *Biochemistry* **1983**, *22*, 2373. (d) Harshman, K. D.; Dervan, P. B. *Nucleic Acids Res.* **1985**, *13*, 4825. (e) Fox, K. R.; Waring, M. J. *Nucleic Acids Res.* **1984**, *12*, 9271. (f) Lane, M. J.; Dobrowiak, J. C.; Vournakis, J. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 3260.

(21) (a) Schultz, P. G.; Taylor, J. S.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 6861. (b) Taylor, J. S.; Schultz, P. G.; Dervan, P. B. *Tetrahedron* **1984**, *40*, 457. (c) Schultz, P. G.; Dervan, P. B. *J. Biomol. Struct. Dyn.* **1984**, *1*, 1133. (d) Wade, W. S.; Mrksich, M.; Dervan, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 8783.

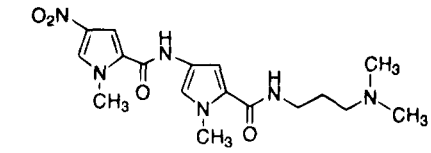
(22) (a) Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1376. (b) Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. *J. Mol. Biol.* **1985**, *183*, 553. (c) Coll, M.; Frederick, C. A.; Wang, A. H.-J.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8385.

(23) (a) Patel, D. J.; Shapiro, L. *J. Biol. Chem.* **1986**, *261*, 1230. (b) Klevitt, R. E.; Wemmer, D. E.; Reid, B. R. *Biochemistry* **1986**, *25*, 3296. (c) Pelton, J. G.; Wemmer, D. E. *Biochemistry* **1988**, *27*, 8088; *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5723.

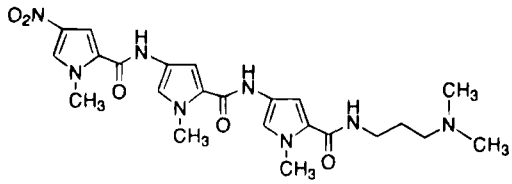
(24) It is also interesting how the compounds **4** and **5** alter the binding specificity. Studies with synthesized molecules bearing pyrrole moiety: (a) Lown, J. W.; Krowicki, K.; Bhat, U. G.; Ward, B.; Dabrowiak, J. C. *Biochemistry* **1986**, *25*, 7408; Lown, J. W.; Krowicki, K.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1986**, *29*, 1210. (b) Kissinger, K.; Krowicki, K.; Dabrowiak, J. C.; Lown, J. W. *Biochemistry* **1987**, *26*, 5590. Korowicki, K.; Lown, J. W. *J. Org. Chem.* **1987**, *52*, 3493. (c) Lee, M.; Chang, D. K.; Hartley, J. A.; Pon, R. T.; Krowicki, K.; Lown, J. W. *Biochemistry*, **1988**, *27*, 445. (d) Wang, W.; Lown, J. W. *J. Med. Chem.* **1992**, *35*, 2890. (e) He, G.-X.; Browne, K. A.; Blaskó, A.; Bruise, T. C. *J. Am Chem. Soc.* **1994**, *116*, 3716. (f) Blaskó, A.; Browne, K. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1994**, *116*, 3726. (g) Blaskóó, A.; Browne, K. A.; He, G.-X.; Bruice, T. C. *J. Am. Chem. Soc.* **1993**, *115*, 7080. (h) Browne, K. A.; He, G.-X.; Bruice, T. C. *J. Am. Chem. Soc.* **1993**, *115*, 7072. (i) He, G.-X.; Browne, K. A.; Groppe, J. C.; Blaskó, A.: Mei, H.-Y.; Bruice, T. C. *J. Am. Chem. Soc.* **1993**, *115*, 7061. (j) Browne, K. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1992**, *114*, 4951. (k) Bruice, T. C.; Mei, H.-Y.; He, G.-X.; Lopez, V. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 1700. (l) Griffin, J. H.; Dervan, P. B. *J. Am. Chem. Soc.* **1986**, *108*, 5008. (m) Youngquist, R. S.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 2565. (n) Anneheim- Herbelin, G.; Perree-Fauvet, M.; Gaudemer, A.; Helissey, P.; Giorgi-Renault, S.; Gresh, N. *Tetrahedron Lett.* **1993**, *34*, 7263. (o) Otsuka, M.; Masuda, T.; Haupt, A.; Ohno, M.; Shiraki, T.; Sugiura, Y.; Maeda, K. *J. Am. Chem. Soc.* **1990**, *112*, 838. (p) Goodsell, D.; Dickerson, R. E. *J. Med. Chem.* **1986**, *29*, 727. (q) Marky, L. A.; Breslaner, K. J. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 4359. (r) Ward, B.; Rehfuss, R.; Goodisman, J.; Dabrowiak, J. C. *Biochemistry* **1988**, *27*, 1198.

(25) (a) Hertzberg, R. P.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 313. (b) Reference **20a**. (c) Reference **20c**. (d) Schultz, P. G.; Dervan, P. B. *J. Am. Chem. Soc.* **1983**, *105*, 7748.

(26) Nishiwaki, E.; Tanaka, S.; Lee, H.; Shibuya, M. *Heterocycles* **1988**, 27, 1945.



6



7

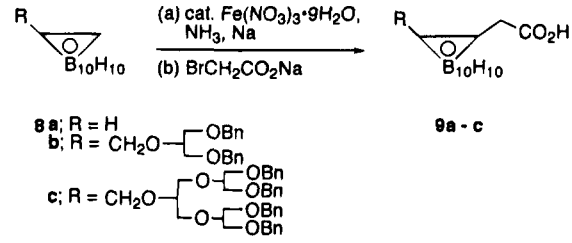
ses of carboranyl acetic acids **9** with (or without) cascade polyol units[27] are shown in Scheme 1. We have developed polyols of a cascade type as a water-solubilizing element.[27] Cascade polyols have no asymmetric centers, so that no diastereoisomers are formed when they are bonded to biologically active chiral molecules. Furthermore, the number of hydroxy groups can be changed at will, and thus a systematic change of water-solubility is attained. The coupling between the carboranyl acetic acids **9a**–**c**[28] and the pyrrol units (**6** and **7**) is shown in Scheme 2. The reduction of the nitro groups of **6** and **7** to the corresponding amines followed by condensation with **9a**–**c** using HOBt and DCC gave the desired coupling products in good to allowable yields; **4a**, 51%; **10b**, 52%; **10c**, 65%; **5a**, 45%; **11b**, 34%; **11c**, 65%. Removal of benzyl groups of **10** and **11** using $Pd(OH)_2$-catalyzed hydrogenation gave the carborane containing netropsin **4b**, **4c** and distamycin derivatives **5b**, **c**; **4b**, 62%; **4c**, 83%; **5b**, 50%; **5c**, 69%. The transformation of the closo-carboranyl cage into its nido-form was not observed on the way to the synthesis of the target molecules. The carboranes bearing netropsin and distamycin derivatives **4a**–**c** and **5a**–**c** are stable in air and easy to be handled.

Footprinting. MPE·Fe(II) footprinting[20c] on the 216 base pair Pvu I/Bam HI restriction fragment from pBLUESCRIPT KS(+1−) (bp 2958) is shown in Figure 1. Lanes 1 and 2 are the products of the Maxam-Gilbert sequencing reactions for G and A+G, respectively, and are used as markers for base identification. Partial nucleotide sequence of the 216 bp Pvu I/Bam HI restriction fragment is shown in Figure 2. Lane 3 is the control experiment in the absence of inhibiting drugs, which contains 100 μM DNA, 10 μM MPE·Fe(II), and 4 mM DTT (dithiothreitol), demonstrating the relatively uniform cleavage pattern generated by MPE·Fe(II). Lane 4 is the positive control experiment in the presence of distamycin A (50 μM). From the DNA cleavage inhibition patterns, it is clear that distamycin A binds not only to the serial A,T base pairs but also to the discrete A,T-rich base pairs at this concentration.

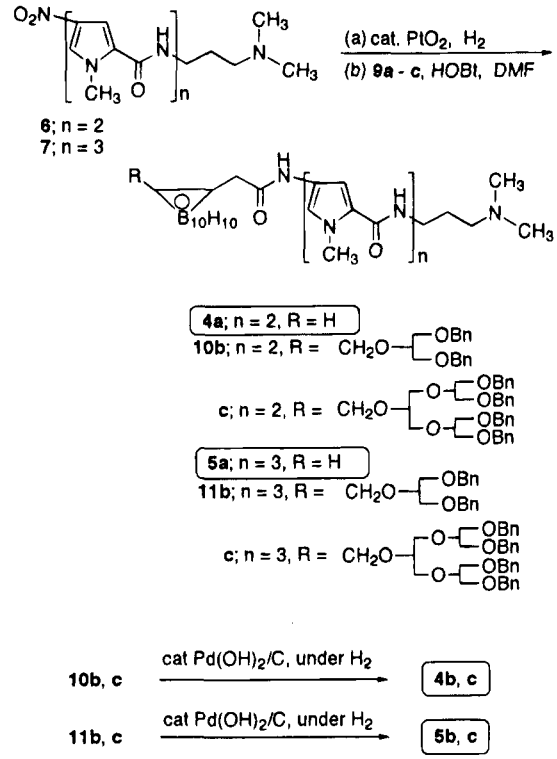
Lanes 5, 6 and 7, 8 are footprinting of the carboranes containing netropsin and distamycin analogues without water-solubilizing moieties (**4a** and **5a**, respectively). Different concentrations of **4a** (500 and 50 μM) and **5a** (500 and 50 μM) were allowed to equilibrate with the 216 base pair DNA fragments (100 μM in base pairs). The DNA cleavage inhibition patterns observed in the gel autoradiogram (lanes 5, 6 and 7, 8) indicates that **4a** slightly binds to the III and IV parts of the DNA fragments which contain five serial A,T base pairs, whereas **5a** binds more selectively than **4a**. The water solubilities of **4** and **5** at 37 °C are as follows; **4a**, 1.75×10^{-4} M; **5a**, 7.58×10^{-5} M; **4b**, 3.33×10^{-4} M; **5b**, 1.89×10^{-4} M; **4c**, 2.25×10^{-3} M; **5c**, 9.81×10^{-4} M. The water solubility increased as the number of hydroxy groups increased, and the three pyrrole ring series **5** exhibited lower solubility than the corresponding two ring series **4**. It was clear that **4c** and **5c** were dissolved at 500 μM (5×10^{-4} M) concentration. However, it was not unambiguous whether **4a**, **4b**, **5a**, and **5b** were dissolved completely at 500 μM concentration or not, although they were dissolved in methanol before incubation (see Experimental Section).

DNA cleavage inhibition patterns of **4b** and **5b**, the carboranes containing netropsin and distamycin derivatives with two hydroxy groups, at the concentrations of 500 and 50 μM are shown in lanes 9, 10 and 11, 12. **4b** binds to the A,T-rich base pairs (III and IV parts), and the comparison of lanes 9, 10 with lanes 5, 6 indicates that **4b** binds more selectively to the base pairs than **4a**. Very interestingly, **5b** binds not only to the III and IV

Scheme 1. Syntheses of Carboranyl Acetic Acids with (or without) Cascade Polyol Units



Scheme 2.



(27) (a) Nemoto, H.; Wilson, J. G.; Nakamura, H.; Yamamoto, Y. *J. Org. Chem.* **1992**, 57, 435. (b) Nemoto, H.; Cai, J.; Yamamoto, Y. *J. Chem. Soc. Chem. Commun.* **1994**, 577.

(28) Haushalter, R. C.; Butler, W. H.; Rudolph, R. W. *J. Am. Chem. Soc.* **1981**, *103*, 2620.

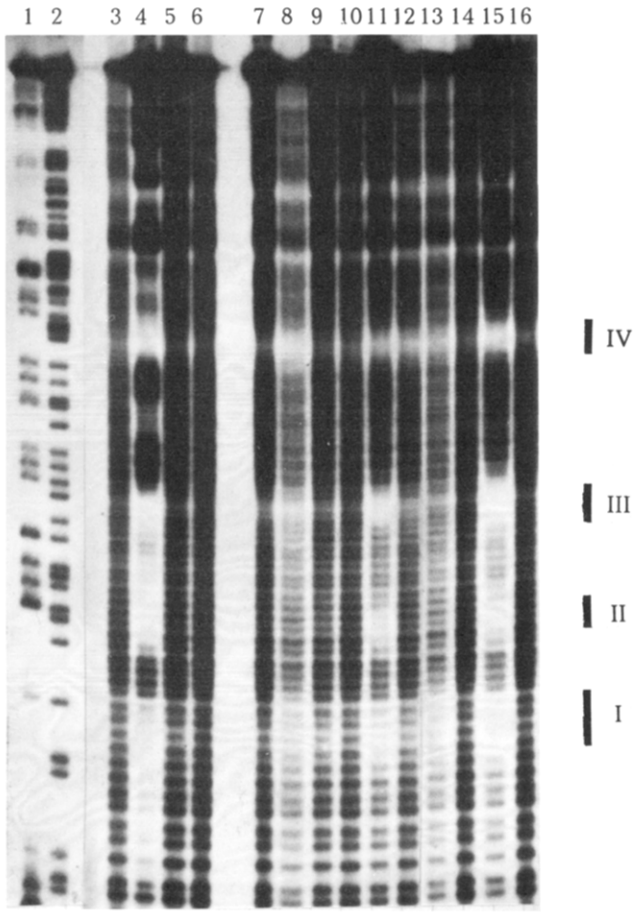


Figure 1. MPE·Fe(II) footprint analysis of the carboranes containing DNA binding compounds to the 5′-labeled 216 bp Pvu I/Bam HI restriction fragment. Lanes 1/2, G and A+G reactions of Maxam-Gilbert sequence determination; 3, reaction without inhibition drug; 4, reaction with distamycin A at 50 μM; lanes 5/6, 7/8, 9/10, 11/12, 13/14, and 15/16 indicate reactions containing compounds **4a**, **5a**, **4b**, **5b**, **4c**, and **5c** at 500 μM and 50 μM, respectively.

parts but also to the I and II parts of the DNA fragments which contain four serial A,T base pairs. Since the water solubilities of **4a**, **4b**, **5a**, and **5b** are less than 5×10^{-4} M (500 μM), they may be past their limit of solubility in spite of the presence of methanol (see Experimental Section) and may present at lower concentrations than 500 μM. Although the water solubility of **5b** is less than that of **4b**, **5b** binds more selectively to the DNA than **4b** at 500 μM (lanes 10 and 12). Therefore, it is concluded that increase of water solubility is not an only important factor for selective binding, but the number of pyrrole rings is also important.

The results on the carboranes containing netropsin and distamycin analogues with four hydroxy groups, **4c** and **5c**, are shown in lanes 13, 14 and 15, 16, respectively. The DNA cleavage inhibition patterns of **4c** (lanes 13 and 14 at 500 μM and 50 μM) showed similar results as those of **4b**; it bound to the IV and III parts. Here again, the comparison of lanes 13, 14 with lanes 9, 10 indicates that **4c** binds more selectively than **4b**. Lane 15 (500 μM) shows that **5c** binds to the I, II, III, and IV parts, and it is clear that **5c** binds more selectively than **5b**. Comparison of lane 15 with lane 4 leads to a conclusion that **5c** binds to the same sites as does distamycin A, although their concentrations are not identical.

In general, the binding to the DNA fragments becomes less selective by attaching a carborane framework to netropsin and distamycin analogues (for example, lanes 5 and 6, 7 and 8, and 9 and 10). However, several interesting and important phenomena can be observed from Figure 1. The compounds **5** bearing three pyrrole rings bind more selectively and preferentially to the A,T-rich base pairs compared with the compounds **4** with two pyrrole rings. Irrespective of **4** or **5**, the increase of the number of hydroxy groups enhances the binding selectivity of the compounds. For example the order of the binding selectivity of **5** is as follows: **5c** > **5b** ≫ **5a**. Accordingly, the hydroxy groups are important not only as a water-solubilizing element of carborane derivatives, but also as a moiety for enhancement of DNA binding selectivity.

In conclusion, we have clarified that **5c** binds to the same sites as does distamycin A and that the hydroxy groups are important not only as a water solubilizing element of carborane derivatives but also as a moiety for enhancement of DNA binding selectivity. It is interesting that **4c** and **5c** can bind to DNA even in the presence of a sterically demanding group such as *o*-carborane. Biological properties of **4** and **5** are now under investigation.

Experimental Section

Synthesis of Carboranes Containing Netropsin and Distamycin Analogues. All melting points were not corrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 400 MHz ^{1}H (67.5 MHz ^{13}C) with tetramethylsilane as an internal standard in CD_3OD. Chemical shifts are given in ppm (δ); in ^{1}H-NMR, d, s, brs, t, qnt, and m indicate doublet, singlet, broad singlet, triplet, quintet, and multiplet, respectively. In ^{13}C-NMR, q, t, d, and s indicate quarternary, methyne, methylene, and methyl, respectively based on the DEPT measurement. Elementary analyses were carried out at the Analytical Center of Tohoku University. In IR data, s and m indicate strong and medium, respectively. Column chromatography was performed on 230−400 mesh silica gel. Syntheses of **6** and **7** were carried out according to the known method.[26] Carboranylacetic acid (**9a**) was synthesized according to the reported procedure.[28]

[2-[[1,3-Bis(benzyloxy)propyloxy]methyl]carboranyl]-acetic Acid 9b. To a solution of catalytic amount $Fe(NO_3)_3 \cdot 9H_2O$ in NH_3 (40−50 mL) at −35 °C was added sodium (458.3 mg, 19.92 mmol) in three portions, and the mixture was stirred for 15 min. A solution of 2-[[1,3-bis(benzyloxy)propyloxy]-methyl]carboranane (**8b**)[28] (4.27 g, 9.96 mmol) in THF (20 mL) was added to the mixture over 2 min, and the mixture was stirred for 30 min at −35 °C. A suspension of bromoacetic acid sodium salt, prepared from bromoacetic acid (2.77 g, 19.94 mmol) and sodium hydride (525.8 mg, 22 mmol) in THF (20 mL) at 0 °C, was added dropwise to the resulting mixture. The mixture was stirred for 30 min at −35 °C and an additional 3 h from −35 °C to room temperature. The reaction mixture was cooled to 0 °C, quenched with HCl(aq) (3 N), and extracted with three portions of ether. The combined organic layers were washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography (hexane:AcOEt:MeOH = 4:4:1) to give **9b** as a colorless gummy oil (3.02 g, 6.18 mmol, 62.1%): IR ($CHCl_3$) 3040m, 2890m, 2600s, 1730s, 1450m, 1100s cm^{-1}; ^{1}H-NMR (CD_3OD) 7.34−7.26 (m, 10H), 4.49 (s, 4H), 4.28 (s, 2H), 3.83−3.72 (m, 1H), 3.63−3.48 (m, 4H), 3.16 (s, 2H); ^{13}C-NMR (CD_3OD): 170.3q, 137.5q, 128.0t, 127.0t, 1287.8t, 79.2t, 78.0q, 74.4d, 73.8q, 71.5d, 71.2d, 41.7d. Anal. Calcd for $C_{22}H_{34}B_{10}O_5$: C, 54.3; H, 7.04. Found: C, 54.02; H, 6.96.

[2-[[1,3-Bis[1,3-bis(benzyloxy)propyloxy-2]propyloxy-2]methyl]carboranyl]acetic Acid 9c. By the same procedure as above, the acid **9c** (5.68 g, 6.97 mmol, 87.9% yield) was synthesized from **8c** (6.0g, 7.93 mmol) as a colorless gummy oil: IR ($CHCl_3$) 3416w, 3060s, 2868s, 2581s, 1736s, 1620s, 1495s, 1453s, 1116s, 697s cm^{-1}. ^{1}H-NMR (CD_3OD) δ 7.35−7.20 (m, 20H), 4.52−4.43 (m, 8H), 4.25 (s, 2H), 3.72−

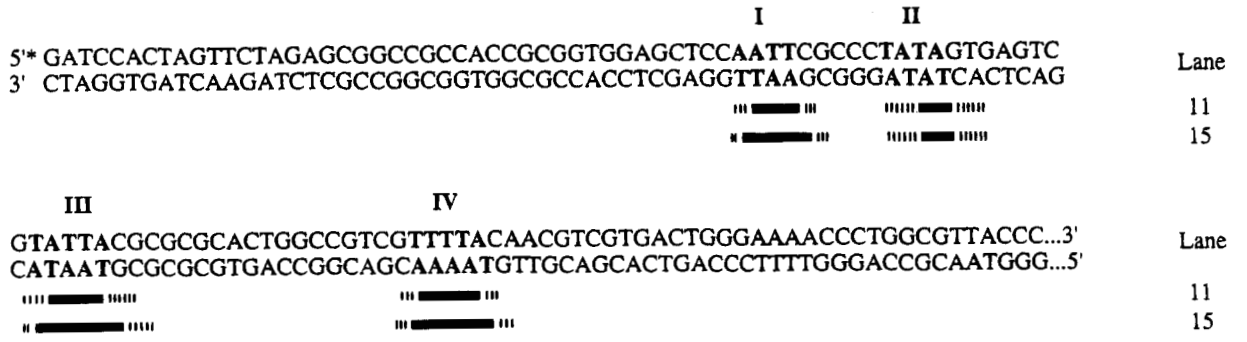


Figure 2. Partial nucleotide sequence of the 216 bp PvuI/BamHI restriction fragment from plasmid pBLUESCRIPT KS detailing four A+T-rich binding sites (bold type). The sequence from left to right corresponds to the bottom to top of the gel autoradiogram in Figure 1, lane 11 (**5b** at 500 μM) and lane 15 (**5c** at 500 μM). The dotted lines and the bold lines indicated the selective bindings of the drugs to the base pairs.

3.44 (m, 15H), 3.13 (s, 2H); ^{13}C-NMR ($CDCl_3$) δ 172.5q, 139.6q, 129.4t, 128.9t, 128.9t, 81.0t, 80.2q, 79.9t, 75.1q, 74.4d, 71.3d, 71.2d, 71.2d, 43.2d. Anal. Calcd for $C_{42}H_{58}B_{10}O_9{}^1/_2H_2O$: C 61.22; H, 7.22. Found: C, 61.03; H, 6.95.

3-[4-[4-(Carboranylacetamido)-1-methylpyrrole-2-carboxamido]-1-methylpyrrole-2-carboxamido]-1-(dimethylamino)propane (4a). A suspension of **6** (49 mg, 0.13 mmol) and PtO_2 (18 mg) in MeOH (15 mL) was stirred vigorously under H_2 at rt for 1 h. The resulting mixture was filtered to remove platinum catalyst. The filtrate was concentrated to remove MeOH. A solution of the residue dissolved in DMF (2 mL) was added to a mixture of **9a** (32 mg, 0.16 mmol), HOBt (18 mg, 0.13 mmol), and DCC (33 mg, 0.13 mmol) in DMF (1.5 mL) at rt. After being stirred for 6 h, the mixture was concentrated to remove DMF. The residue was purified by column chromatography (MeOH:concd NH_3(aq) = 99:1) to give **4a** as a light yellow solid (35 mg, 0.0659 mmol, 51% yield): mp 132–4 °C; IR (KBr) 3250s, 2920s, 2550s, 1620s, 1420s, 1245s, 1000m cm^{-1}; ^{1}H-NMR (CD_3OD) 7.15 (d, J = 2.0 Hz, 2H), 6.84 (d, J = 2.0 Hz, 1H), 6.78 (d, J = 2.0 Hz, 1H), 4.77 (brs, 1H), 3.88 (s, 3H), 3.85 (s, 3H), 3.33–3.28 (m, 2H, overlapped with CHD_2OD), 3.25 (s, 2H), 2.47–2.38 (m, 2H), 2.28 (s, 6H), 1.77 (qnt, J = 7.0 Hz, 2H); ^{13}C-NMR (CD_3OD) 165.3q, 164.1q, 161.1q, 124.8q, 124.6q, 123.2q, 122.4q, 120.7t, 120.4t, 106.1t, 105.9t, 71.9q, 61.6t, 58.3d, 45.4s, 44.3d, 38.5d, 36.9s, 36.7s, 28.2d. Anal. Calcd for $C_{21}H_{38}B_{10}N_6O_3.H_2O$: C, 45.97; H, 7.35; N, 15.32. Found: C, 46.15; H, 7.04; N, 15.53.

3-{4-[4-[4-(Carboranylacetamido)-1-methylpyrrole-2-carboxamido]-1-methylpyrrole-2-carboxamido]-1-methylpyrrole-2-carboxamido-1-(dimethylamino)propane (5a). **5a** (491 mg, 0.732 mmol, 44.8% yield) was obtained as a light yellow solid from **7** (837 mg, 1.68 mmol) and **9a** (339 mg, 1.67 mmol) by the same procedure as above: mp 178–180 °C; IR (KBr) 3280m, 2950m, 2560m, 1630s, 1430s, 1400s, 1260s, 1100m cm^{-1}; ^{1}H-NMR (CD_3OD) 7.18–7.12 (m, 3H), 6.91(d, J = 2.0 Hz, 1H), 6.86 (d, J = 2.0 Hz, 1H), 6.78 (d, J = 2.0 Hz, 1H), 4.76 (brs, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 3.33–3.27 (m, 2H, overlapped with CHD_2OD), 3.24 (s, 2H), 2.48–2.40 (m, 2H), 2.29 (s, 6H), 1.83–1.68 (m, 2H). ^{13}C-NMR (CD_3OD) 172.7q, 165.4q, 164.2q, 161.3q, 124.8q, 124.6q, 124.5q, 123.3q, 122.9q, 122.4q, 120.8t, 120.8t, 120.5t, 106.5t, 106.2t, 106.0t, 71.9q, 61.5t, 58.1d, 45.2s, 44.3d, 38.3d, 36.9, 36.8, 36.8s, 28.0d. Anal. Calcd for $C_{27}H_{44}B_{10}N_8O_4{\cdot}H_2O$: C, 48.34; H, 6.91; N, 16.7. Found: C, 48.33; H, 6.59; N, 16.43.

3-[4-[4-[[1,3-Bis(benzyloxy)propoxy-2]methylcarboranylacetamido]-1-methylpyrrole-2-carboxamido]-1-methylpyrrole-2-carboxamido]-1-(dimethylamino)propane (10b). **10b** (456 mg, 0.56 mmol, 52.3% yield) was obtained as a light yellow solid from **6** (440 mg, 1.17 mmol) and **9b** (520 mg, 1.07 mmol) by the same procedure as above: mp 79–81 °C; IR (KBr) 3270m, 3030w, 2945m, 2860m, 2550m, 1630s, 1570s, 1510s, 1430s, 1400s, 1250s, 1110s cm^{-1}; ^{1}H-NMR (CD_3OD) 7.33–7.21 (m, 10H), 7.16 (d, J = 2.0 Hz, 1H), 7.10 (d, J = 2.0 Hz, 1H), 6.85 (d, J = 2.0 Hz, 1H), 6.80 (d, J = 2.0 Hz, 1H), 4.49 (s, 4H), 4.36 (s, 2H), 3.87 (s, 3H), 3.84 (s, 3H), 3.84–3.76 (m, 1H), 3.66–3.50 (m, 4H), 3.38–3.26 (m, 2H), 3.17 (s, 2H), 2.47–2.36 (m, 2H), 2.27 (s, 6H), 1.76 (qnt, J = 7.0 Hz, 2H); ^{13}C-NMR (CD_3OD) 165.5q 164.8q, 161.0q, 139.3q, 129.4t, 128.9t, 128.8t, 125.4q, 125.4q, 123.2q, 122.6q, 120.7t, 120.1t, 106.1t, 106.0t, 80.2t, 79.9q, 74.7q, 74.1d, 71.6d, 71.1d, 58.3d, 45.4s, 42.9d, 38.6d, 36.9s, 36.8s, 28.2d. Anal. Calcd. for $C_{39}H_{58}B_{10}N_6O_6.H_2O$: C, 56.23; H, 7.26; N, 10.09. Found: C, 56.5; H, 7.09; N, 10.46.

3-{4-[4-[[1,3-Bis[1,3-bis(benzyloxy)propyloxy-2]propyloxy-2]methylcarboranylacetamido]-1-methylpyrrole-2-carboxamido]-1-methylpyrrole-2-carboxamido}-1-(dimethylamino)propane (10c). By the same procedure as above, **10c** (400 mg, 0.35 mmol, 65% yield) was obtained from **6** (223 mg, 0.6 mmol) and **9c** (439 mg, 0.54 mmol) as a light yellow solid: mp 30–32 °C; IR (KBr) 3300s, 2900s, 2580s, 1640s, 1530s, 1460s, 1100s cm^{-1}; ^{1}H-NMR (CD_3OD): δ 7.30–7.20 (m, 20H), 7.12 (d, J = 2.0 Hz, 1H), 7.10 (d, J = 2.0 Hz, 1H), 6.90 (d, J = 2.0 Hz), 6.78 (d, J = 2.0 Hz, 1H), 4.44 (s, 4H), 4.43 (s, 4H), 4.27 (s, 2H) 3.84 (s, 3H), 3.79 (s, 3H), 3.73–3.44 (m, 15H), 3.32–3.28 (m, 2H, overlapped with CHD_2OD), 3.12 (s, 2H), 2.45–2.35 (m, 2H), 2.26 (s, 6H), 1.75 (qnt, 2H, J = 7.0 Hz); ^{13}C-NMR (CD_3OD) δ 165.1q, 164.1q, 161.1q, 139.4q, 129.4t, 128.8t, 128.7t, 124.8t, 124.6t, 123.2t, 122.6t, 120.7q, 120.3q, 106.2q, 106.0q, 80.3t, 80.0q, 79.8t, 74.6q, 74.5d, 71.3d, 71.0d, 70.8d, 58.3d, 45.6s, 43.0d, 38.6d, 36.9s, 36.8s, 28.2d. Anal. Calcd for $C_{59}H_{82}B_{10}N_6O_{10}{\cdot}H_2O$: C, 61.01; H, 7.29; N, 7.24. Found: C, 61.08; H, 7.29; N, 7.40.

3-{4-[4-[4-[[1,3-Bis(benzyloxy)propoxy-2]methylcarboranylacetamido]-1-methylpyrrole-2-carboxamido]-1-methylpyrrole-2-carboxamido]-1-methylpyrrole-2-carboxamido}-1-(dimethylamino)propane (11b). **11b** (784 mg, 0.821 mmol, 33.6% yield) was obtained from **7** (1218 mg, 2.44 mmol) and **9b** (1189 mg, 2.44 mmol) as a light yellow solid by the same procedure as above: mp 114–6 °C; IR (KBr) 3255s, 2920s, 2860s, 2550s, 1625s, 1420s, 1590s, 1245s, 1090s cm^{-1}; ^{1}H-NMR (CD_3OD) 7.32–7.20 (m, 10H), 7.17 (d, J = 2.0 Hz, 1H), 7.16 (d, J = 2.0 Hz), 7.10 (d, J = 2.0 Hz, 1H), 6.94 (d, J = 2.0 Hz, 1H), 6.87 (d, J = 2.0 Hz, 1H), 6.81 (d, J = 2.0 Hz, 1H), 4.48 (s, 4H), 4.35 (s, 2H) 3.87 (s, 6H), 3.84 (s, 3H), 3.84–3.76 (m, 1H), 3.65–3.50 (m, 4H), 3.36–3.26 (m, 2H) 3.16 (s, 2H), 2.57–2.38 (m, 2H), 2.31 (s, 6H), 1.77 (qnt, J = 7.0 Hz, 2H); ^{13}C-NMR (CD_3OD) 174.1q, 165.3q, 164.2q, 162.6q, 139.3q, 128.9t, 128.8t, 128.6t, 124.6q, 124.6q, 124.5q, 123.3q, 123.3q, 122.2q, 120.9t, 120.6t, 106.6t, 106.2t, 106.2t, 80.2t, 79.9q, 74.8q, 74.3d, 71.5d, 71.2d, 58.0d, 45.1s, 43.3d, 38.3d, 37.0s, 36.9s, 36.9s, 28.0d. Anal. Calcd for $C_{45}H_{64}B_{10}N_8O_7.H_2O$: C, 56.59; H, 6.96; N, 11.73. Found: C, 56.26; H, 6.89; N, 11.75.

3-{4-[4-[4-[[1,3-Bis[1,3-bis(benzyloxy)propyloxy-2]-propyloxy-2]methylcarboranylacetamido]-1-methylpyrrole-2-carboxamido]-1-methylpyrrole-2-carboxamido]-1-methylpyrrole-2-carboxamido}-1-(dimethylamino)propane (11c). By the same procedure as above, **11c** (572 mg, 0.45 mmol, 64% yield) was obtained from **7** (384 mg, 0.77 mmol) and **9c** (570 mg, 0.7 mmol) as a light yellow solid: mp 58–60 °C; IR (KBr) 3300s, 2940s, 2860s, 2570s, 1640s, 1580s, 1450s, 1400s, 1275s, 1090s cm^{-1}; ^{1}H-NMR (CD_3OD) δ 7.28–7.26 (m, 20H), 7.16 (d, J = 2.0 Hz, 1H), 7.13 (d, J = 2.0 Hz,

1H), 7.10 (d, J = 2.0 Hz, 1H), 6.92 (d, J = 2.0 Hz, 2H), 6.80 (d, J = 2.0 Hz, 1H), 4.44 (s, 4H), 4.43 (s, 4H), 4.27 (s, 2H), 3.87 (s, 3H), 3.84 (s, 3H), 3.79 (s, 3H), 3.72–3.46 (m, 15H), 3.32–3.27 (m, 2H, overlapped with CHD_2OD), 3.12 (s, 2H), 2.47–2.37 (m, 2H), 2.27 (s, 6H), 1.76 (qnt, 2H, J = 7.0 Hz); ^{13}C-NMR (CD_3OD) δ 165.3q, 164.2q, 161.3q, 161.2q, 139.5t, 129.4t, 128.9t, 128.7t, 124.9t, 124.7t, 124.7t, 123.3t, 123.3t, 122.6t, 120.8q, 120.8q, 120.4q, 106.6q, 106.2q, 106.1q, 80.4t, 79.8t, 79.7q, 74.6q, 74.4d, 71.2d, 71.1d, 70.8d, 58.4d, 45.4s, 43.1d, 38.6d, 36.9s, 36.8s, 36.8s, 28.2d Anal. Calcd. for $C_{65}H_{88}B_{10}N_8O_{11}\cdot^1/_2H_2O$: C, 61.25; H, 7.04; N, 8.79. Found: C, 61.24; H, 6.81; N, 8.79.

3-[4-[4-[[1,3-Dihydroxypropoxy-2]methylcarboranylacetamido]-1-methylpyrrole-2-carboxamido]-1-methylpyrrole-2-carboxamido]-1-(dimethylamino)propane (4b). A solution of **10b** (1.487 g, 1.78 mmol) in MeOH (5 mL) in the presence of concd HCl(aq) (4 drops) and suspended $Pd(OH)_2$/C (30 mg) was stirred under hydrogen atmosphere at rt for 20 h. The resulting suspension was quenched with Et_3N (0.1 mL) and filtered to remove palladium catalyst. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography (MeOH:concd NH_3(aq) = 98:2) to give **4b** (739 mg, 1.10 mmol, 61.8% yield) as a light yellow solid: mp 133–5 °C; IR (KBr) 3270s, 2950m, 2575m, 1630s, 1580s, 1520s, 1435s, 1400s, 1260m, 1100m cm^{-1}; ^{1}H-NMR (CD_3OD) 7.16 (d, J = 2.0 Hz), 6.87 (d, J = 2.0 Hz, 1H), 6.80 (d, J = 2.0 Hz, 1H), 4.39 (s, 2H) 3.89 (s, 3H), 3.86 (s, 3H), 3.76–3.48 (m, 5H), ~3.3 (s, 2H, dipped into CHD_2OD), 3.34–3.29 (m, 2H, dipped into CHD_2OD), 2.53–2.43 (m, 2H), 2.33 (s, 6H), 1.79 (qnt, J = 7.0 Hz, 2H); ^{13}C-NMR (CD_3OD) 165.4q, 164.4q, 161.3q, 124.9q, 124.5q, 123.3q, 122.6q, 120.5t, 120.3t, 106.2t, 106.1t, 83.6t, 80.1q, 74.8q, 71.5d, 62.4d, 58.0d, 44.9s, 42.9d, 38.2d, 36.9s, 36.8s, 27.8d. Anal. Calcd for $C_{25}H_{46}B_{10}N_6O_6.2H_2O$: C, 44.76; H, 7.51; N, 12.53. Found: C, 45.15; H, 7.2; N, 12.31.

3-{4-[4-[[1,3-Bis[1,3-dihydroxypropyloxy-2]propyloxy-2]methylcarboranylacetamido]-1-methylpyrrole-2-carboxamido]-1-methylpyrrole-2-carboxamido}-1-(dimethylamino)propane (4c). By the same procedure as above, **4c** (208 mg, 0.266 mmol, 83% yield) was obtained from **10c** (366 mg, 0.32 mmol) as a light yellow solid: mp 110–2 °C; IR (KBr) 3250s, 2950m, 2575m, 1640s, 1530s, 1430s, 1260s, 1100s, 1050s cm^{-1}; ^{1}H-NMR (CD_3OD) δ 7.18 (d, 1H, J = 2.0 Hz), 7.15 (d, J = 2.0 Hz, 1H), 6.90 (d, J = 2.0 Hz, 1H), 6.79 (d, J = 2.0 Hz, 1H), 4.41 (s, 2H), 3.89 (s, 3H), 3.85 (s, 3H), 3.79–3.52 (m, 15H), 3.48–3.38 (m, 2H), ~3.3 (s, 2H, dipped in CD_2HOD), 2.51–2.40 (m, 2H), 2.31 (s, 6H), 1.78 (qnt, 2H, J = 7.0 Hz); ^{13}C-NMR (CD_3OD) δ 165.5q, 164.4q, 161.3q, 124.8t, 124.5t, 123.3t, 122.6t, 120.9q, 120.5q, 106.3q, 106.1q, 83.1t, 80.5t, 80.0q, 74.9q, 71.4d, 70.8d, 62.5d, 58.0d, 45.0ds, 43.1d, 38.1d, 36.9s, 36.8s, 27.9d. Anal. Calcd for $C_{31}H_{58}B_{10}N_8O_{10}\cdot H_2O$: C, 46.49; H, 7.55; N, 10.49. Found: C, 46.51; H, 7.25; N, 10.46.

3-{4-[4-[4-[[1,3-Dihydroxypropoxy-2]methylcarboranylacetamido]-1-methylpyrrole-2-carboxamido]-1-methylpyrrole-2-carboxamido]-1-methylpyrrole-2-carboxamido}-1-(dimethylamino)propane (5b). 5b (500 mg, 0.63 mmol, 50%) was obtained from **11b** (1.20 g, 1.26 mmol) as a light yellow solid: mp 141–3 °C; IR (KBr) 3280s, 2950m, 2560s, 1635s, 1580s, 1520s, 1430s, 1400s, 1250s, 1200m, 1100m cm^{-1}; ^{1}H-NMR (CD_3OD) 7.19–7.15 (m, 3H), 6.93 (d, J = 2.0 Hz, 1H), 6.89 (d, J = 2.0 Hz, 1H), 6.79 (d, J = 2.0 Hz, 1H), 4.39 (s, 2H) 3.90 (s, 6H), 3.86 (s, 3H), 3.74–3.50 (m, 5H), 3.36–3.20 (m, 4H, dipped into CHD_2OD), 2.52–2.40 (m, 2H,), 2.31 (s, 6H), 1.78 (qnt, J = 7.0 Hz, 2H); ^{13}C-NMR (CD_3OD) 165.5q, 164.3q, 161.5q, 161.5q, 124.9q, 124.7q, 124.7q, 123.3q, 123.29q, 122.64q, 120.8t, 120.8t, 120.5t, 106.6t, 106.2t, 106.1t, 83.7t, 80.2q, 74.9q, 71.5d, 62.5d, 58.3d, 45.3d, 43.0d, 38.5d, 36.9s, 36.8s, 36.8s, 28.2d. Anal. Calcd for $C_{31}H_{52}B_{10}N_8O_7.2H_2O$: C, 46.96; H, 7.12; N, 14.13. Found: C, 46.79; H, 6.89.

3-{4-[4-[4-[[1,3-Bis[1,3-Dihydroxypropyloxy-2]propyloxy-2]methylcarboranylacetamido]-1-methylpyrrole-2-carboxamido]-1-methylpyrrole-2-carboxamido]-1-methylpyrrole-2-carboxamido}-1-(dimethylamino)propane (5c). 5c (237 mg, 0.26 mmol, 69% yield) was obtained from **11c** (485 mg, 0.38 mmol) as a light yellow solid: mp 123–5 °C; IR (KBr) 3300m, 2910w, 2550w, 1638s, 1520s, 1430s, 1250m, 1100m cm^{-1}; ^{1}H-NMR (CD_3OD) δ 7.18 (d, 2H, J = 2.0 Hz), 7.16 (d, J = 2.0 Hz, 1H), 6.92 (d, J = 2.0 Hz, 1H), 6.91 (d, J =2 Hz, 1H), 6.78 (d, J = 2.0 Hz, 1H), 4.41 (s, 2H), 3.90 (s, 3H), 3.89 (s, 3H), 3.86 (s, 3H), 3.80–3.50 (m, 15H), 3.49–3.37 (m, 2H), ~3.3 (s, 2H, dipped in CD_2HOD), 2.47–2.37 (m, 2H), 2.28 (s, 6H), 1.77 (qnt, 2H, J = 7 Hz); ^{13}C-NMR(CD_3OD) δ 165.5q, 164.2q, 161.4q, 161.3q, 124.8q, 124.6q, 124.6q, 123.3q, 123.2q, 122.6t, 122.6t, 120.9t, 120.4t, 106.5t, 106.1t, 106.1t, 83.0t, 80.5t, 80.0q, 74.8q, 71.3d, 70.7d, 62.4d, 58.2d, 45.3s, 43.1d, 38.5d, 36.9s, 36.8s, 36.8s, 28.2d. Anal. Calcd for $C_{37}H_{64}B_{10}N_8O_{11}\cdot H_2O$: C, 48.14; H, 7.21; N, 12.14. Found: C, 47.87; H, 7.12; N, 12.35.

Footprinting.[20c] Distamycin A was a commercial product. Stock solutions (5 mM) were prepared by dissolving the antibiotic, **4**, and **5** in methanol and stored at −20 °C. MPE was given by Prof. Dervan. MPE was dissolved in water, and the stock solution (1.0 mM) was stored at −20 °C. MPE·Fe(II) solution was prepared, just prior to use, by mixing the MPE stock solution with a 1.0 mM stock solution of $Fe(NH_4)_2(SO_4)_2\cdot 6H_2O$. Radiolabeled DNA fragments for this investigation were a gift from Mr. A. Kobayashi of the biochemistry laboratory in our department. The plasmid pBLUESCRIPT KS (+1−) (bp 2958) was cleaved with the restriction enzyme Pvu I and Bam HI, and the 216 base pair fragments were radiolabeled at the 5' end with [^{32}P]d ATP. The resulting DNA fragments were stored in a 36 mM NH_4OAc/3mM EDTA solution at −20 °C. DNA cleavage reactions with MPE were carried out in the following way. Each reaction initially consisted of a 2 μL solution of 50 mM Tris (pH 7.5), 250 mM NaCl, and 0.5 mM DNA base pairs composed of 5'-^{32}P end-labeled restriction fragment and carrier calf thymus DNA. To this was added 1 μL of a solution of each compound in methanol. This was incubated for 30 min at room temperature. The cleavage reaction was initiated by the addition of 5 μL of 20 μM MPE·Fe(II) (freshly prepared from stocks before use) and 2 μL of freshly prepared 20 mM dithiothreitol solution. Final concentrations in the 10 μL reaction volume were 10 mM Tris (pH 7.5), 50 mM NaCl, 4 mM dithiothreitol, 3.6 mM NH_4OAc, 0.3 mM EDTA, 100 μM DNA base pairs and 10 μM MPE·Fe(II). Antibiotic concentrations (50–500 μM) are described in the Figure 1 caption. Each reaction was allowed to run at 37 °C for 15 min and stopped by freezing in liquid N_2, salts were removed with 70% EtOH as in the usual method, and then the gels were freeze-dried under vacuum and resuspended on a formamide loading buffer for gel electrophoresis (without removal of salts, the bands of autoradiogram were broaded).

Acknowledgment. We acknowledge Professor P. B. Dervan for providing us with MPE. We thank Professor Y. Fujii, Assoc. Professor K. Sogawa, and Mr. A. Kobayashi for their help on the footprinting experiment. This work was supported by the joint studies program (1994–1995) of the institute for molecular science.

JO950202R