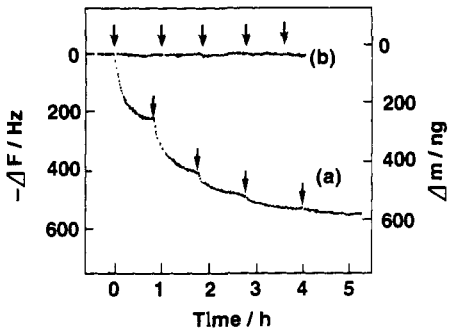

Figure 1. Typical frequency changes of 10-mer nucleotide (106 ng, 35 pmol) immobilized QCM, responding to each addition (120 ng, 0.05 pmol) of (a) M13 phage DNA and (b) nonsite M13 phage DNA in a 10-mL aqueous solution at 50 °C.

a handmade oscillator designed to drive the quartz at its resonance frequency in aqueous solutions.[6,7] The frequency changes were followed by universal frequency counter (Iwatsu Co., Tokyo, Japan, Model SC 7201) attached to a microcomputer system (NEC Co., Tokyo, Japan, Model PC 9801). Calibration showed that a frequency decrease of 1 Hz corresponded to a mass increase of 1.05 ng on the QCM electrode (16 mm^2) both in aqueous solutions and in air.[6,7]

We have prepared the 10-mer deoxynucleotide having a mercaptopropyl group at the 5'-phosphate end by the phosphoramidite method[8] as a probe DNA, whose sequence was complementary with the *Eco*RI binding site of single-stranded M13 phage DNAs (7249 base pairs, MW = 2.4×10^6). Nonsite phage M13 DNAs (7239 base pairs) were also prepared in which the complementary 10 nucleotides were removed. The QCM was immersed in an aqueous solution of the probe DNA with an SH group (1500 ng in 5 mL) for 20 min at 25 °C.[9] The amount of the probe nucleotide immobilized was calculated to be 106 ± 10 ng (35 pmol) on both sides (16 $mm^2 \times 2$) of the electrodes. This value was calculated to be ca. 8% coating coverage of the Au electrode surface of the QCM.

Figure 1 shows typical time courses of frequency changes of the probe nucleotide-immobilized QCM responding to each addition of M13 phage DNA or nonsite M13 DNA as a target DNA to distilled water (10 mL) at 50 °C. In the case of the complementary M13 DNA, the frequency decreased with time, responding to each addition, and it was saturated at $-\Delta F = 560 \pm 10$ Hz (mass increase of 590 ± 10 ng) at a concentration of 600 ng (0.25 pmol) in 10 mL. At the saturation point, all DNAs added in the solution bound to the probe nucleotide on the QCM, and an M13 phage DNA was calculated to hybridize with ca. 0.5 mol % of probe nucleotides on the QCM. The minimum concentration needed to detect M13 phage DNA was observed to be 1 pM (25 ng in 10 mL) in the solution. The nonsite M13 phage DNA hardly interacted with the probe DNA on the QCM at 50 °C (see curve b in Figure 1).

After the hybridization of the probe nucleotide on the QCM with M13 phage DNA at 20 °C, the temperature of the solution was increased gradually at the rate of 10 °C/min. The frequency of $-\Delta F = 560 \pm 10$ Hz due to the hybridization increased discontinuously between 55 and 65 °C and reverted to the original value of the probe-immobilized QCM ($\Delta F = 0$ Hz) above 70 °C. This phenomenon occurred reversibly when the temperature was decreased to 20 °C. These results indicate that the hybridization on the QCM was melted (separated) with increasing temperature in the range 55-65 °C, and T_m of the hybridization was determined to be 60 °C.

Table I. Hybridization Amounts between the Probe-Immobilized QCM and Various 10-mer Nucleotides in Solution at 25 °C[a]

nucleotides[b]	Δm/ng	% hybridization[c]
3'dCCCTTAAGCA5'	380 ± 10	100
3'dCCCTTAAGGG5'	350 ± 10	92
3'dTGCTTAAGCA5'	350 ± 10	92
3'dCCCTAAAGCA5'	125 ± 10	31
3'dCCCTGAAGCA5'	100 ± 10	26
3'dCTGCTACGGG5'	0	0
3'dAGCCGTACCC5'	0	0

[a] The probe nucleotide of HS-5'PdGGGAATTCGT3' was immobilized on the QCM with a relatively large amount (847 ng, 280 pmol, ca. 65% coating coverage of electrodes) in order to get high sensitivity for the detection of low molecular weight oligonucleotides, compared with M13 phage DNA. [b] Concentration: 400 ng (130 pmol) in 10 mL. The complementary sequences with the probe are shown with underlines. [c] Indicates how much of the nucleotide in the solution bound to the probe on the QCM.

The QCM technique could be applied for the detection of hybridization with various 10-mer oligonucleotides as target DNAs. Hybridization amounts obtained from frequency changes are summarized in Table I. In the case of the totally complementary 10-mer nucleotide, all target nucleotides added in the solution bound and hybridized on the QCM. The oligonucleotides having more than eight continuous complementary base pairs could also bind with the probe on the QCM. This hybridization was not stable, since the frequency reverted to the original value when the QCM moved to the new aqueous solution. Oligonucleotides having five continuous and complementary base pairs partially bound with the probe, but the hybridization was not stable.

In summary, the oligonucleotide-immobilized QCM will become a new useful tool to detect specific DNAs by the weight of the direct hybridization in solutions without any pre- or posttreatments and without nonspecific bindings of DNAs and proteins. We emphasize that this method is also useful to study molecular level kinetic understanding of base-pair hybridizations in oligonucleotides.

(7) Calibration was performed by the deposition of polymer or lipid cast film[6a] or Langmuir-Blodgett lipid film[6c] on the QCM, and the constant obtained was consistent with the Sauerbrey equation constant[5] both in air and the aqueous phase.

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Complexation of Benzamidinium by a New Family of Artificial Receptors

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In comparison to numerous reports relating the structures of drugs to their interactions with natural recognition sites,[1,2] there are relatively few examples of the complexation of drugs (e.g., barbiturates) by *synthetic* receptors.[3] Benzamidine groups are attractive targets for complexation studies because they are found

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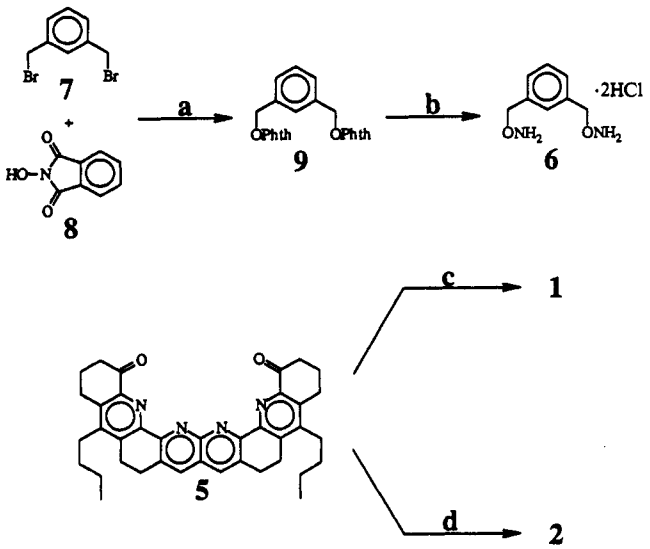
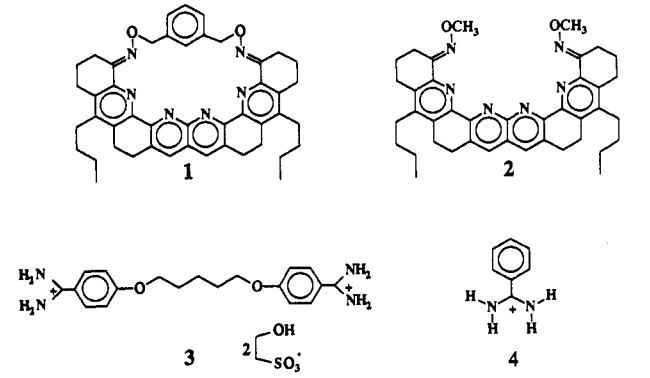


Figure 1. Synthesis of benzamidine receptors **1** and **2**: (a) Et_3N, CH_3CN, reflux (66%); (b) H_2NNH_2, EtOH, reflux/HCl (86%); (c) **6**, EtOH (50%); (d) CH_3ONH_2·HCl, EtOH (65%).

in a number of important antibacterial and antiprotozoal agents.[4] We are particularly interested in developing a synthetic receptor capable of recognizing pentamidine isethionate (**3**), an important DNA-binding bis(benzamidine) drug used for treatment of *Pneumocystis carinii* in AIDS patients.[5] In this communication we describe the synthesis and complexation properties of two new artificial receptors (**1** and **2**) that bind benzamidinium (**4**) through a network of convergent hydrogen bonds and/or π-stacking interactions.



The new receptors were synthesized by the methods shown in Figure 1. Double condensation reactions between the previously reported diketone **5**[6] and bis(*O*-alkylhydroxylamine) **6** or commercially available *O*-methylhydroxylamine hydrochloride gave **1** or **2**, respectively. Compound **6** was prepared in two steps from α,α'-dibromo-*m*-xylene (**7**) and *N*-hydroxyphthalimide (**8**) by a variation of the Gabriel synthesis used for the preparation of *O*-benzylhydroxylamine.[7] Macrocyclization of diketone **5** with **6** gave receptor **1** in good yield without need of high-dilution conditions. Analytically pure samples of **1** and **2** containing 1.5–2.0 water molecules of hydration were obtained by recrystallization and drying at 78 °C in vacuo.[8]

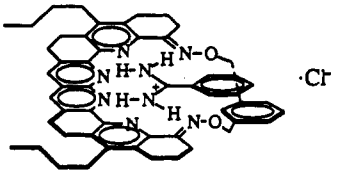


Figure 2. Host–guest orientation proposed for **1**·benzamidine hydrochloride complex.

Receptors **1** and **2**, as well as diketone **5**, form 1:1 complexes with benzamidinium salts, as demonstrated by solid–liquid extraction experiments. When excess solid benzamidine hydrochloride was added to CD_2Cl_2 solutions of **1**, **2**, or **5**, approximately 1 equiv of the salt was extracted, according to integration of the ^1H NMR signals of host and guest. The aromatic and benzylic protons of the bridge of **1** are shifted approximately 0.6 ppm upfield, and the N–H protons of the guest appear at low field (9.0, 9.6 ppm). These observations are consistent with the structure of the complex designed by examination of CPK models (Figure 2). In this structure the meta-bridged benzene ring lies below the cleft formed by the fused pyridine and naphthyridine rings of **1**. Rapid exchange and ring inversion on the ^1H NMR time scale apparently causes the signals of the diastereotopic benzylic protons of the bridge to coalesce to a broad singlet at room temperature. The methoxy protons of **2** are shifted 0.9 ppm upfield in the complex, suggesting that the two receptors adopt similar conformations. Both 1:1 complexes were also isolated as crystalline monohydrates. While X-ray crystal structures of neither complex have been obtained, the solubilization of benzamidinium chloride[9] and observed ^1H NMR shifts are qualitative evidence of host–guest complexation in the predicted manner.

The complexation of benzamidinium by **1** and **2** may also be detected by UV–visible spectroscopy. Addition of benzamidine hydrochloride to dilute solutions of **1** or **2** in CH_2Cl_2/EtOH, 95:5 (v/v), resulted in bathochromic shifts of the absorption maxima from 390 to 396 nm and from 388 to 396 nm, respectively. Subsequent experiments in which a solution of **1** (1.63×10^{-5} M, CH_2Cl_2/EtOH, 95:5 (v/v)) was titrated with benzamidine hydrochloride (2.93×10^{-6}–1.46×10^{-4} M) revealed its stability constant, K_s, to be $5 \pm 1 \times 10^5$ M^{-1}.[10] A similar titration experiment with **2** (1.52×10^{-6} M, CH_2Cl_2/EtOH, 95:5 (v/v)) produced a UV–visible absorption plot demonstrating complete complexation throughout the titration. A minimum stability constant of 2×10^7 M^{-1} can be calculated, accounting for experimental error. The large difference in the two K_s values suggests that the aromatic moiety may partially block the binding cleft in one or more conformations of receptor **1**. Guest binding may require a greater degree of conformational reorganization in **1** than in **2**, but the design of **1** requires potentially useful π-contact between the aromatic rings of host and guest.

These preliminary results show that readily synthesized receptors can form strong complexes with benzamidines. Hosts based on hexagonal lattice receptors[6,11] contrast with other approaches to complexation of organic molecules, such as molecular clefts derived from Kemp's triacid,[12] molecular tweezers,[13] diacetylene-bridged naphthalenophanes,[14] and more flexible π-stacking receptors.[15] Artificial receptors with multiple binding

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sites and responsive chromophores or fluorophores constitute a feasible approach to measurement of bis(benzamidine) drugs, such as pentamidine. Sensors and reagents based on this approach could prove superior to conventional chromatographic methods for analysis of therapeutic drugs.[4a,16]

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Supplementary Material Available: UV-visible data presented as tables and graphs of absorbance vs guest:host ratio for titration of **1** and **2** with benzamidine hydrochloride and experimental procedures and characterization data for compounds **1**, **2**, **5**, **6**, **9** and complexes of **1** and **2** with benzamidine hydrochloride (8 pages). Ordering information is given on any current masthead page.

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Hydrogen Pentazole: Does It Exist?

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Pentazoles (RN_5) present a long history of controversy regarding their stability and isolation dating back to 1915 when Lipschitz reported the synthesis of the silver salt, AgN_5.[1] This was promptly refuted by Curtius et al.[2] who stated: "A repetition of Lipschitz's experiments has shown that all his observations ... are erroneous and that there is no formation of pentazoles." Further attempted syntheses were not successful until 1957, when Huisgen and Uzi reported the synthesis of the phenylpentazole derivative in a paper entitled, "Pentazoles I. The Solution of the Classical Problem of Organic Nitrogen Chemistry".[3] X-ray crystal structures for this species have also been determined;[4] however, no synthesis of the HN_5 pentazole prototype has ever been reported. Pentazoles are directly analogous to the cyclopentadienyl systems, and theoretical work has examined the nature of the anion (N_5^-)[5] and metal-ring complexes.[6] As part of a continuing investigation of nitrogen-based metastable species, Bartlett and co-workers have investigated the properties of N_3H_3,[7] N_4, N_6, and N_8,[8] as have others.[9] Theory predicts that N_3H_3, tetrahedral N_4, and octahedral N_8 are stable minimia for their respective potential energy surfaces, but experimental observation will be difficult and none has been reported.

In this paper, we present the structural and vibrational properties of the synthetic target and several novel pentazole derivatives calculated using coupled-cluster (CC) and many-body perturbation theory (MBPT) theory.[10] We use the ACES II program system[11] and DZP basis sets.[12] Our goal is to provide accurate structural, energetic, and spectral information as a prelude to potential experimental observation.

In Figure 1, we present the structural parameters for hydrogen pentazole (HN_5) and several potential derivatives, including the unusually interesting N_5-N_5 bipentazole molecule. Electron correlation effects lengthen most bonds and diminish the degree of bond alternation, suggesting greater aromaticity. The CCSD N-N bond lengths average 1.33 Å, being intermediate between ordinary N-N single, 1.40-1.47 Å, and double bonds, 1.20-1.24

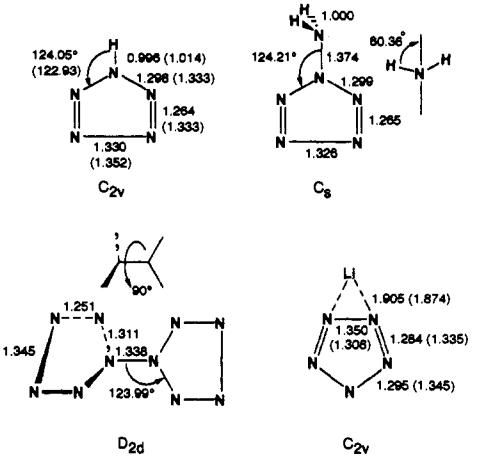

Figure 1. Molecular structure of pentazole and its derivatives determined at the SCF and MBPT(2) (in parentheses) levels. Values are given in angstroms and degrees. For hydrogen pentazole, CCSD results are 1.329, 1.304, and 1.360 Å for bonds, 1-2, 2-3, and 3-4 respectively. The NH bond is 1.010 Å.

Table I. SCF and Correlated Vibrational Analysis for Pentazole (HN_5)

	SCF/DZP			MBPT(2)/DZP		
mode	sym	freq (cm^{-1})	IR intensity (km/mol)	sym	freq (cm^{-1})	IR intensity (km/mol)
1	b_1	666	(81.1)	b_1	636	(43.3)
2	b_1	817	(42.8)	b_1	743	(67.1)
3	a_2	841	(0.0)	a_2	745	(0.0)
4	a_1	1132	(14.8)	b_2	1026	(13.8)
5	b_2	1177	(20.8)	a_1	1050	(4.2)
6	a_1	1239	(0.3)	a_1	1083	(0.6)
7	b_2	1273	(17.1)	a_1	1148	(0.1)
8	a_1	1399	(7.6)	b_2	1160	(22.6)
9	a_1	1485	(3.9)	a_1	1193	(3.2)
10	b_2	1516	(14.5)	b_2	1252	(3.9)
11	b_2	1719	(1.4)	b_2	1465	(13.7)
12	a_1	3922	(193.1)	a_1	3700	(149.6)

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