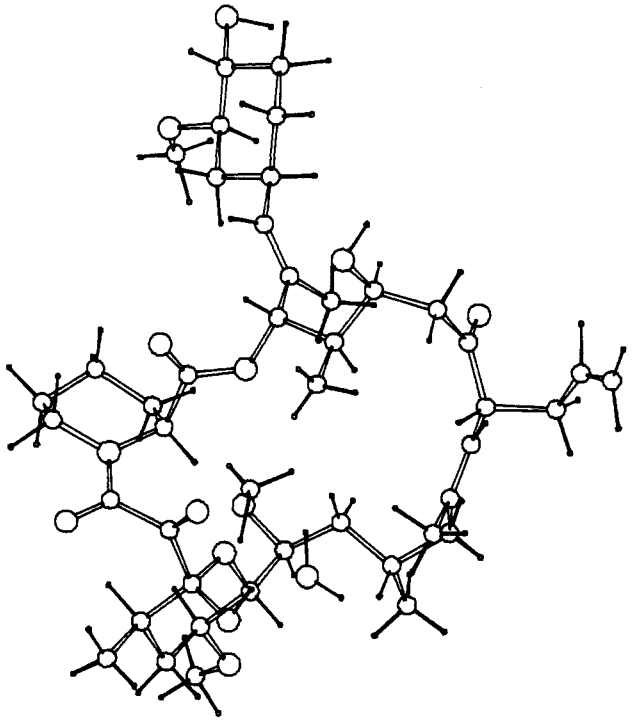

**Figure 1.** A perspective drawing of the X-ray model of FK506 (**1**). The water molecule is included.

to the full structure of FK506. The geometry of the two trisubstituted olefins in **1** was assigned to both to be E on the basis of the upfield resonations of the Me groups bonded to these double bonds (19-Me, $\delta_c$ 15.8; 27-Me, $\delta_c$ 13.9). Since several attempts to assign the stereochemistry of the other functional groups were unsuccessful, an X-ray analysis was performed on crystalline FK506 itself (Figure 1),[10] establishing the relative stereochemistry as depicted in **1**. The absolute configuration was determined by the fact that **1** contains L-pipecolic acid (see above). The tautomeric equilibration of **1** in solution might be associated with a restricted rotation of the amide bond within the macrolide ring.[11,12]

FK506 represents a new class of macrolide lactones with amino acid and hemiketal-masked $\alpha,\beta$-diketoamide functionalities incorporated in a 23-membered ring.[13] The activity of FK506 was considerably greater than that of cyclosporin A in various immunosuppression assays.[14]

(10) Crystal data for **1** ($C_{44}H_{69}NO_{12}\cdot H_2O$, $M$ = 804.0): orthorhombic; space group $P2_12_12_1$; unit cell $a$ = 10.939 (1) Å, $b$ = 15.878 (1) Å, $c$ = 27.184 (1) Å; $v$ = 4721.0 Å$^3$; $Z$ = 4; Dx = 1.131 g·cm$^{-3}$. Intensities were measured on a Rigaku AFC-5RU diffractometer by using graphite-monocromated Cu K$\alpha$ radiation ($\lambda$ = 1.5418 Å). Of 4484 independent reflections with $2\theta <$ 130°, 4249 were used for structure determination. The structure was determined by direct methods (RANTAN) and successive Fourier syntheses and block-diagonal least-squares. The final $R$ factor, based on the used reflections, was 0.071.

(11) In comparison of the $^{13}$C NMR signals of the major and minor isomers, the most significant differences in chemical shift were observed at C-2 and C-6. In the major isomer, C-2 resonated by 4.2 ppm to lower fields than in the minor isomer, while C-6 was observed by 4.7 ppm to upper fields, suggesting that in the major isomer the amide bond is in cis conformation in accord with the result of the X-ray crystal analysis.

(12) The $^{13}$C NMR spectrum in solid state revealed that FK506 exists as one conformer (cis amide conformation): see the Supplementary Material.

(13) The closest literature analogue that contains these functionalities is rapamycin, which has been described as an antifungal antibiotic: Findlay, J. A.; Radics, L. *Can. J. Chem.* **1980**, *58*, 579.

(14) The exceptional activity of FK506 will be reported separately. (a) Kino, T.; Hatanaka, H.; Miyata, S.; Inamura, N.; Yajima, T.; Goto, T.; Okuhara, M.; Kohsaka, M.; Aoki, H.; Ochiai, T. *J. Antibiot.*, in press. (b) Inamura, N.; Nakahara, K.; Kino, T.; Goto, T.; Aoki, H.; Yamaguchi, I.; Kohsaka, M.; Ochiai, T. *Transplantation*, in press. (c) Ochiai, T.; Nakajima, K.; Nagata, M.; Hori, S.; Asano, T.; Isono, K. *Transplantation*, in press. (d) Ochiai, T.; Nagata, M.; Nakajima, K.; Suzuki, T.; Sakamoto, K.; Enomoto, K.; Gunji, Y.; Uematsu, T.; Goto, T.; Hori, S.; Kenmochi, T.; Nakagouri, T.; Asano, T.; Isono, K.; Hamaguchi, K.; Tsuchida, H.; Nakahara, K.; Inamura, N.; Goto, T. *Transplantation*, in press.

**Supplementary Material Available:** Spectral data (IR, $^1$H NMR, and $^{13}$C NMR) for **1**, **2**, **3**, **6**, **7**, and **9** and additional X-ray crystallographic data for **1** (9 pages). Ordering information is given on any current masthead page.

## Molecular Recognition: Hydrogen Bonding and Stacking Interactions Stabilize a Model for Nucleic Acid Structure

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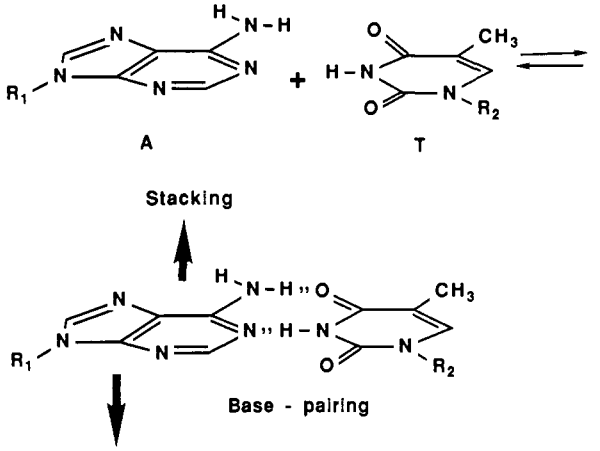
The classical form of molecular recognition is the base pairing within nucleic acids as formulated by Watson and Crick.[1] The complementary hydrogen bonding surfaces shown in eq 1 for




(1)

adenine (A) and thymine (T) provide a vehicle for information transfer, while stacking interactions between adjacent base pairs provide additional stability for the helical structure.[2] The hydrogen bonding aspects of eq 1 have been examined in detail by Rich[3] and Hammes[4] with use of derivatives of A and T in the noncompeting solvent $CDCl_3$, while the stacking of individual bases in $H_2O$ was observed by Chan.[5] Here, we introduce a model system in which both forces can operate simultaneously.

The new models, e.g., **1**, are designed in accord with the principles of molecular recognition[6] and feature stacking and hydrogen bonding surfaces that converge on the substrate from perpendicular directions. Moreover, their bulk reduces the dimerization (self-recognition) that is generally observed[3] in addition to eq 1. The scaffolding for the new structures is provided by derivatives of Kemp's triacid[7] **2**, in which the U-shaped relationship that exists between any two carboxyl functions is enforced by the equatorial methyl groups. Sublimation of **2** or its successive treatment with $(CF_3CO)_2O$ and water gives the anhydride acid[7] **3a**. With $NH_4OH$ **3a** gives the imide acid[8] **3b** (mp > 280 °C) from which the acid chloride **3c** (mp 171 °C) can be obtained with $SOCl_2$. The new amides are obtained by acylation of the aromatic amines **4a–e** with **3c**. In addition, the methyl ester **3d**

(1) Watson, J. D.; Crick, F. H. C. *Nature (london)* **1953**, *171*, 737–8.

(2) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; Chapter 6.

(3) Kyogoku, Y.; Lord, R. G.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *57*, 250–257. Iwahaski, H.; Kyogoku, Y. *J. Am. Chem. Soc.* **1977**, *99*, 7765.

(4) Hammes, G. C.; Park, A. C. *J. Am. Chem. Soc.* **1968**, *90*, 4151–57.

(5) Chan, S. I.f Schweitzer, M. P.; Ts'o, P. O. P.; Helmkamp, G. K. *J. Am. Chem. Soc.* **1964**, *86*, 4182. Schweitzer, M. P.; Chan, S. I.; Ts'o, P. O. P. *J. Am. Chem. Soc.* **1965**, *87*, 5241.

(6) Rebek, J., Jr. *Science (Washington, DC)* **1987**, *235*, 1478–1484.

(7) Kemp, D. S.; Petrakis, K. S. *J. Org. Chem.* **1981**, *46*, 5140–43.

(8) All new compounds were characterized by 300-MHz PMR, 75-MHz $^{13}$C NMR, and FTIR spectroscopy. Elemental analyses were either within 0.3% of calculated combustion values or within 0.001 of calculated mass spectral values.

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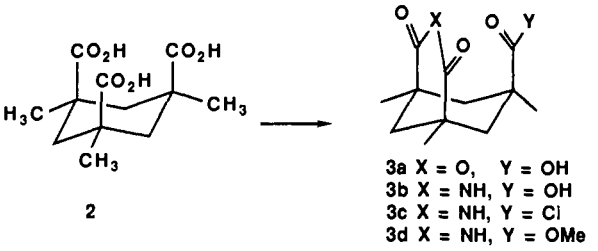
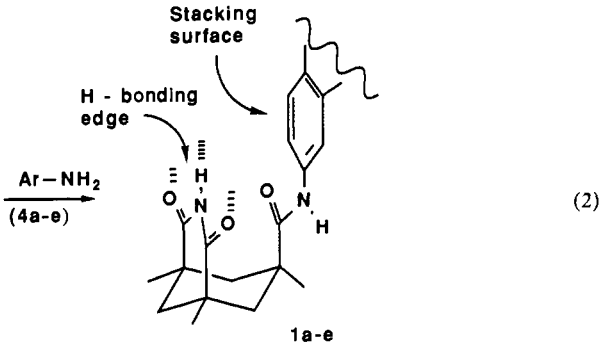
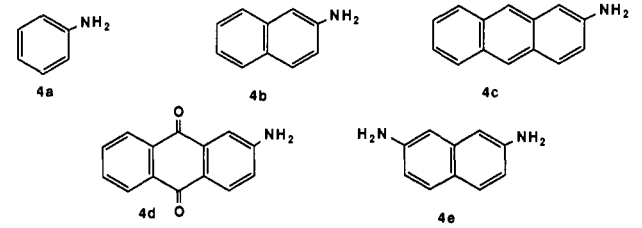


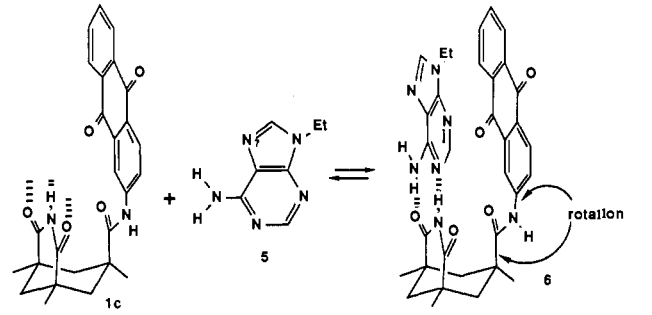




(2)

(mp > 300 °C) can be prepared from **3c** and MeOH. Specific amides prepared were **1a** (mp 245 °C) from aniline, **1b** (mp 270 °C) from β-naphthylamine (**4b**), **1c** (mp 249–252 °C) from 2-aminoanthracene (**4c**), **1d** (>240 °C, d) from 2-aminoanthraquinone (**4d**), and the bisamide **1e** (mp 205 °C) from 2,7-diaminonapthalene[9] (**4e**).




Complex formation between the imides **1a–e** or **3d** and 9-ethyladenine (**5**) was determined by NMR. In $CDCl_3$ titration of **1** with **5** caused downfield shifts of the imide N–H resonance (7.2 → 12.4 ppm) and adenine $NH_2$ resonances (5.5 → 5.9 ppm) but *upfield* shifts of the aromatic resonances.[10] These spectroscopic changes indicate that hydrogen bonding brings the aromatic nuclei into close proximity as suggested in eq 3 for the anthraquinone complex **6**. Titration data for the various aromatic derivatives as well as the methyl ester **3d** were converted to association constants by using Eadie plots,[11] and these are reported in Table I.



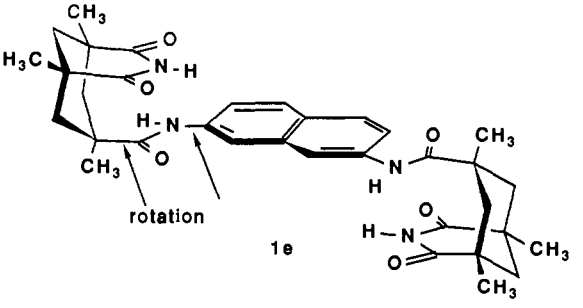

(3)

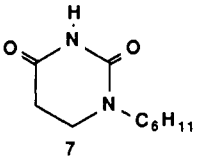
Table I. Titration Data for the Derivatives of the Following Reaction

**1a–e + 5** ⇄ complexes

| amide of | $K_a$, $M^{-1}$ (25 °C, $CDCl_3$) | amide of | $K_a$, $M^{-1}$ (25 °C, $CDCl_3$) |
|---|---|---|---|
| **4a** | 100 | **4d** | 240 |
| **4b** | 120 | **4e** (bisamide) | 11000 (30% $CD_3CN$) |
| **4c** | 440 | **3d** | 75 |

These association constants may be compared with the reference reaction of eq 1. For $R_1$ = Et **5** and $R_2$ = cyclohexyl $K_a$ = 100 was reported while with **5** and the dihydrouracil **7**, $K_a$ = 30.[3] The




entry for **3d** falls within these values, and 75 $M^{-1}$ may be used as the baseline figure to calculate the hydrogen-bonding contribution in the new complexes as 2.5 kcal/mol. Both the Watson–Crick mode shown in eq 3 and the Hoogsteen mode[2] (not shown) involving the amino group and $N_7$ of adenine are expected to contribute to the overall hydrogen bonding.

The effects of both surface size and dipole are revealed in Table I. Rotations about the bonds shown lead to different conformations and various degrees of overlap between aromatic subunits of the adenine and receptor. The small contributions to binding in **1a** and **1b** suggest ineffective overlap in these cases, whereas the anthracene derivative **1c** enjoys better overlap. The nearly sixfold increase in binding over **3d** corresponds to about 1 kcal/mol contributed by stacking with the anthracene surface. The anthraquinone **1d**, a structure related to known intercalaters of double-stranded DNA,[12] is less effective in the context of this model system.

The bisamide **1e** offers two sites for binding adenine. Titration experiments showed only 1:1 complex formation, and the large association constant 1.1 × $10^4$ is consistent with simultaneous Watson–Crick and Hoogsteen base pairing and stacking with the naphthalene surface as shown in **8** (eq 4). Indeed, CPK models show excellent complementary in size, shape, and functionality in the molecular recognition of adenine by **1e**. The chelation provided by the convergent imides in **8** also contributes to the kinetic stability of this complex. Dynamic NMR experiments established $\Delta G_c^{\ddagger}$ = 12.4 kcal/mol at the coalescence temperature $T_c$ = 219 °C for eq 4. For the other cases of Table I, experiments at low temperatures failed to freeze out the exchange reactions, a result anticipated by the low activation barriers observed[4] for the base-pairing reaction of eq 1.

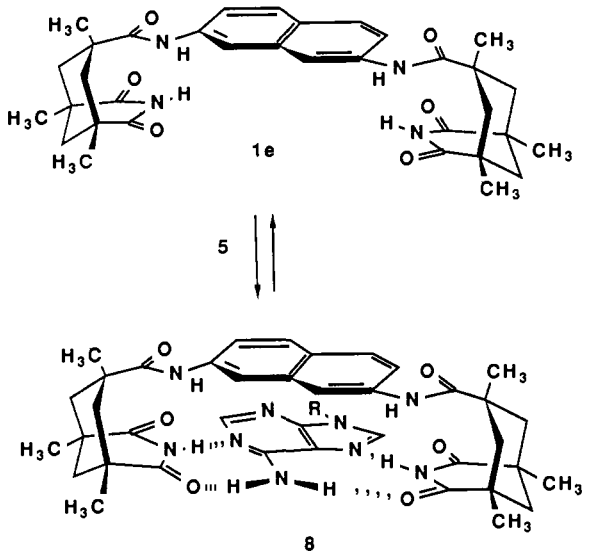
In summary, the new systems provide a realistic model for the intermolecular forces present in double-stranded nucleic acids. Moreover, they show that a combination of weak intermolecular

(9) Bucherer, H. T. *J. Prakt. Chem.* **1904**, *2*, 69, 49. Drake, N. L. In *Organic Reactions*; Vol. I, pp 105–128.

(10) NMR chemical shift changes (ppm) observed on complex formation were as follows: **1a**, 7.1 → 6.7 ($H_4$); **1b**, 7.8 → 7.4 ($H_2$); **1c**, 7.3 → 7.1 ($H_3$); **1d**, 8.2 → 7.9 ($H_2$); **1e**, 7.8 → 7.4 ($H_2$).

(11) Eadie, G. S. *J. Biol. Chem.* **1942**, *146*, 85–93. Hofstee, B. H. J. *Nature (London)* **1959**, *184*, 1296–98.

(12) Quigley, G. J.; Wang, A. H.-J.; Ughetto, G.; van der Marcel, G.; van Boom, J. H.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 7204–7208.




(4)

forces can result in high selectivity in model systems for molecular recognition.

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## Induced Fit in Synthetic Receptors: Nucleotide Base Recognition by a "Molecular Hinge"

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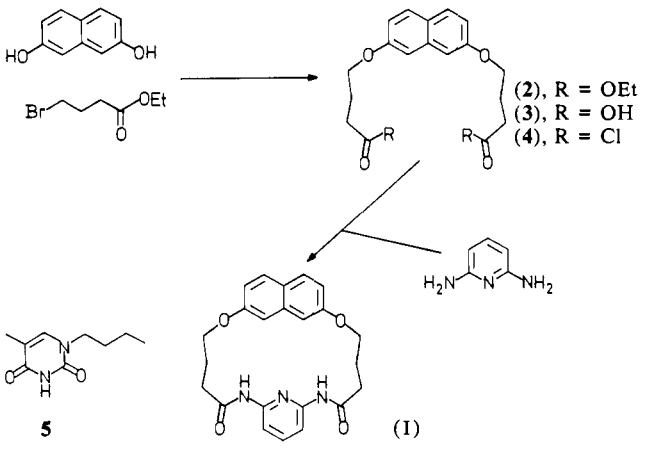
The development of molecules that recognize and bind to specific nucleotides or nucleotide base pairs provides an important goal in contempory bioorganic chemistry.[1] A key element in the design of such specific receptors concerns the incorporation of several recognition features (e.g., hydrogen bonding, hydrophobic or electrostatic) that complement the chemical characteristics of the target. This multiple point binding is dramatically seen in the enzyme ribonuclease $T_1$ which binds its nucleotide substrate via both hydrogen bonding and hydrophobic interactions.[2] In addition to two hydrogen bonds, formed between N-1 and O-6 of the guanine and two amide groups on the peptide backbone, hydrophobic stacking occurs between the aromatic nucleotide base and a tyrosine residue (Tyr 45).[3] Similarly many natural[5] and synthetic[6] DNA binding molecules and artificial DNA cleaving agents[7] employ both intercalation (hydrophobic) and hydrogen bonding interactions.

As part of a program aimed at the preparation of synthetic receptors for biologically active molecules[8] we have sought to incorporate multiple recognition sites into a new class of nucleic acid binding molecules.[9] Our strategy is to assemble hydrogen bonding and hydrophobic groups (and ultimately electrostatic or reactive groups) within a macrocyclic structure that can form a cavity complementary to the nucleotide base substrate. We report here the synthesis, structure, and complexation properties of a macrocycle **1**, containing 2,6-diamidopyridine and naphthalene components, that shows two-point binding to thymine derivatives. Host **1** was also designed to test the possibility of inducing a conformational change on substrate binding which would place a naphthalene ring directly above the bound substrate.

Reaction of 2,7-naphthalene diol with ethyl 4-bromobutyrate ($K_2CO_3$, acetone, reflux) gave diester **2**, (83% yield) which was




then hydrolyzed (acetone, HCl) to diacid **3** (98% yield). Treatment of **3** with oxalyl chloride ($CH_2Cl_2$) afforded diacid chloride **4** which was not isolated but directly cyclized under high dilution conditions ($CH_2Cl_2$, $Et_3N$) with 2,6-diaminopyridine[10] to form macrocyclic host **1** (26% yield).[11] The structure of **1** was confirmed by single-crystal X-ray analysis (Figure 1a) which shows an open conformation with the naphthalene poised away from the pyridine ring at an inter-plane angle of 127.5°. In addition the amide hydrogens lie in the plane of the pyridine and project under the naphthalene ring to provide a partially organized substrate binding region.

Treatment of a $CDCl_3$ solution of **1** with 1 equiv of 1-butylthymine **5**[12,13] results in several characteristic changes in the $^1H$ NMR spectrum.[14] The NH protons on both **1** and **5** are shifted

(1) For general reviews, see: Dervan, P. D. *Science (Washington, DC)* **1986**, *232*, 464. Wakelin, I. P. G. *Med. Res. Rev.* **1986**, *6*, 275. Neidle, S. *Prog. Med. Chem.* **1979**, *16*, 151.

(2) Heinemann, U.; Saenger, W. *Nature (London)* **1982**, *299*, 27.

(3) Distance between parallel guanine and Tyr 45 is 3.5 Å.[2] The electron density map of ribonuclease $T_1$[4] shows an alternative, nonstacking position for Tyr 45. This is probably due to the unbound form and suggests that on substrate binding a conformational change in the enzyme occurs to swing Tyr 45 into a stacking position. Thus, in addition to playing a role in recognition Tyr 45 also acts to lock in or "gate" the substrate.

(4) Heinemann, U.; Wernitz, M.; Pahler, A.; Saenger, W.; Menke, G.; Ruterjans, H. *Eur. J. Biochem.* **1980**, *109*, 109.

(5) For example, actinomycin see Sobell et al., (Sobell, H. M.; Jain, S. C. *J. Mol. Biol.* **1972**, *68*, 28.) or daunomycin see Quigley et al. (Quigley, G. J.; Wang, A. H.-J.; Ughetto, G.; van der Marel, G.; van Boom, J. H.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 7204.).

(6) For example, mitoxantrone, see: Kotovych, G.; Lown, J. W.; Tong, J. P. K. *J. Biomolec. Struct. Dyn.* **1986**, *4*, 111. Islam, S. A.; Neidle, S.; Gandecha, B. M.; Partridge, M.; Patterson, L. H.; Brown, J. R. *J. Med. Chem.* **1985**, *28*, 857.

(7) Dervan, P. D.; Sluka, J. P. *Proceedings of the International Kyoto Conference on Organic Chemistry*; Elsevier: Amsterdam, 1986; p 307.

(8) Hamilton, A. D.; Kazanjian, P. *Tetrahedron Lett.* **1985**, 5735. Mann, M.; Hamilton, A. D.; Pant, N. *J. Chem. Soc., Chem. Commun.* **1986**, 158.

(9) For other synthetic hosts containing more than a single recognition site see, for example: Sheridan, R. E.; Whitlock, H. W., Jr. *J. Am. Chem. Soc.* **1986**, *108*, 7120. Willner, I.; Goren, Z. *J. Chem. Soc., Chem. Commun.* **1983**, 1469. Tabushi, I.; Shimizu, N.; Sugimoto, T.; Shiozuka, M.; Yamamura, K. *J. Am. Chem. Soc.* **1977**, *99*, 7100. Rebek, J.; Nemeth, D. *J. Am. Chem. Soc.* **1986**, *108*, 5637. Kimura, E.; Fujioka, H.; Kodama, M. *J. Chem. Soc., Chem. Commun.* **1986**, 1158. Schmitchen, F. P. *J. Am. Chem. Soc.* **1986**, *108*, 8249.

(10) Freshly sublimed. For another macrocycle containing 2,6-diaminopyridine units, see: Weber, E.; Vogtle, F. *Liebig Ann. Chem.* **1976**, 891.

(11) **1**: $^1H$ NMR ($CDCl_3$) 7.98 (2 H, d, $J$ = 8 Hz, py 3, 5 H), 7.75 (1 H, t, $J$ = 8 Hz, py 4 H), 7.74 (2 H, br s, NH), 7.69 (2 H, d, $J$ = 8 Hz, naph 4, 5 H), 7.11 (2 H, d, $J$ = 2 Hz, naph 1, 8 H), 7.05 (2 H, dd, $J$ = 2, 8 Hz, naph 3, 6 H), 4.30 (4 H, t, $J$ = 6.5 Hz, $CH_2O$), 2.52 (4 H, m. $CH_2CO$), 2.25 (4 H, m, $CCH_2C$).

(12) Prepared by alkylating thymine with butyl bromide ($Me_2SO$, $K_2CO_3$), see: Browne, D. T. In *Synthetic Procedures in Nucleic Acid Chemistry*; Zorbach, W. W., Tipson, R. S., Eds.; Interscience: New York, 1968; p 98.

(13) **5**: $^1H$ NMR ($CDCl_3$) 8.02 (1 H, br s, NH), 7.00 (1 H, s, th 6 H), 3.71 (2 H, t, $J$ = 6.5 Hz, $NCH_2$), 1.93 (3 H, s, ring $CH_3$), 1.65 (2 H, m, $NCH_2CH_2$), 1.36 (2 H, m, $CH_3CH_2$), 0.96 (3 H, t, $J$ = 8 Hz, $CH_2CH_3$).

(14) Complex (1:1) between **1** and **5**: $^1H$ NMR ($CDCl_3$) 10.58 (1 H, br s, th NH), 9.92 (2 H, br s, py NH), 8.05 (2 H, d, $J$ = 8 Hz, py 3, 5 H), 7.79 (1 H, t, $J$ = 8 Hz, py 4 H), 7.46 (2 H, d, $J$ = 8 Hz, naph 4, 5 H), 7.86 (4 H, m, naph 1, 3, 6, 8 H), 6.71 (1 H, s, th H), 4.20 (4 H, t, $J$ = 4.5 Hz, $OCH_2$), 3.47 (2 H, t, $J$ = 8 Hz, th $NCH_2$), 2.64 (4 H, m, $CH_2CO$), 2.24 (4 H, m, $CCH_2C$), 1.74 (3 H, s, th ring $CH_3$), 1.55 (2 H, m, th $NCH_2CH_2$), 1.30 (2 H, m, $CH_3CH_2$), 0.96 (3 H, t, $J$ = 8 Hz, $CH_2CH_3$).

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