Kinetic importance of conformations of nicotinamide adenine dinucleotide in the reactions of dehydrogenase enzymes

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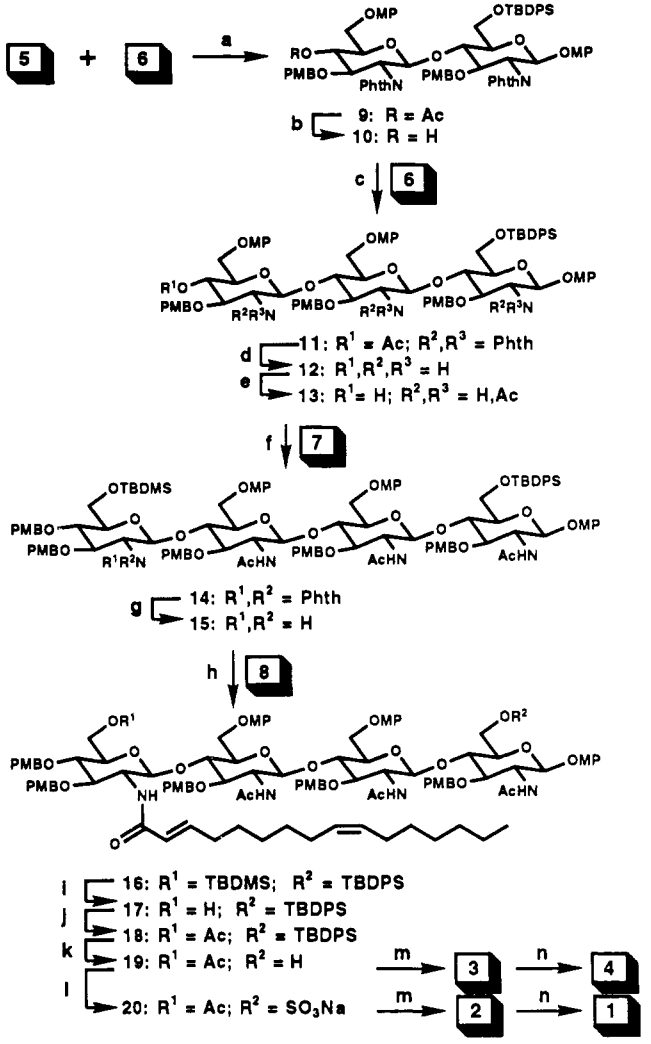
Scheme II. Total Synthesis of NodRm-IV Factors[a]



[a] Reagents and conditions: (a) 1.0 equiv of **5**, 1.75 equiv of **6**, 5.0 equiv of AgOTf, 5.0 equiv of Cp_2ZrCl_2, 1.0 equiv of 2,6-di-*tert*-butyl-4-methylpyridine, 4-Å molecular sieves, CH_2Cl_2, 0–25 °C, 16 h, 56% (plus 36% recovered **5**); (b) 1.0 equiv of K_2CO_3, MeOH–THF (1:1), 25 °C, 2 h, 90%; (c) 2.0 equiv of **6**, 5.0 equiv of AgOTf, 5.0 equiv of Cp_2HfCl_2, 1.0 equiv of 2,6-di-*tert*-butyl-4-methylpyridine, 4-Å molecular sieves, CH_2Cl_2, 0–25 °C, 16 h, 60% (plus 37% recovered **10**); (d) excess of hydrazine hydrate, EtOH–benzene (20:1), 100 °C, 16 h; (e) excess of Ac_2O, MeOH–CH_2Cl_2 (1:1), 25 °C, 30 min, 72% for two steps; (f) 5.0 equiv of **7**, 5.0 equiv of AgOTf, 5.0 equiv of Cp_2HfCl_2, 0.2 equiv of 2,6-di-*tert*-butyl-4-methylpyridine, 4-Å molecular sieves, CH_2Cl_2, 25 °C, 16 h, 50% (plus 25% recovered **13**); (g) excess of hydrazine hydrate, EtOH, 100 °C, 6 h, 87%; (h) 3.0 equiv of **8**, 3.0 equiv of 2-chloro-1-methylpyridinium iodide, 3.3 equiv of Et_3N, MeCN, 25 °C, 2 h, 73%; (i) 1.3 equiv of pyridinium *p*-toluenesulfonate, EtOH, 25 °C, 16 h; (j) 1.5 equiv of Ac_2O, 1.1 equiv of Et_3N, DMAP (cat.), CH_2Cl_2, 25 °C, 10 min, 72% for two steps; (k) 3.0 equiv of TBAF, THF, 25 °C, 1.5 h, 88%; (l) excess of SO_3·NMe_3, pyridine, 25 °C, 1 h, 85%; (m) 20.0 equiv of ceric ammonium nitrate (CAN), MeCN–H_2O (4:1), 25 °C, 1 h, 30%; (n) excess of NaOMe, MeOH, 25 °C, 3 h, 75%.

iodide.[9] Selective removal of the *tert*-butyldimethylsilyl group from compound **16** proceeded smoothly on exposure to PPTS[10] to afford **17**. Acetylation of **17** followed by desilylation with $^nBu_4NF$ gave compound **19**. Sequential deprotection of **19** with ceric ammonium nitrate (CAN) and NaOMe led to the targeted NodRm-IV (Ac) (**3**)[11] and NodRm-IV (**4**), respectively. Alternatively, sulfation of **19** with SO_3·NMe_3 and ion exchange (Na^+) gave compound **20**. Sequential deprotection of **20** under the above conditions gave NodRm-IV (Ac,S) (**2**)[11] and NodRm-IV (S) (**1**). Final products **1–4** were purified by reverse-phase HPLC as described in the supplementary material.

The described chemistry renders these scarce bioactive compounds readily available for further biological studies. Molecular design and structure–activity studies are also now feasible, and so is the isolation of the receptors of these compounds.

Acknowledgment. We thank Drs. Dee H. Huang and Gary Siuzdak, of The Scripps Research Institute, for their NMR and mass spectroscopic assistance, respectively. Stimulating discussions with Professor J. Denarie are also acknowledged. This work was financially supported by the National Institutes of Health and The Scripps Research Institute. D.R.C. thanks Merck Sharp and Dohme for a postdoctoral fellowship (1992).

Supplementary Material Available: Schemes for the synthesis of building blocks **5–8**, including reagents, conditions and yields, and listing of selected physical data for compounds **9**, **11**, **14**, **16**, **19**, **20**, **4**, **3**, **2**, and **1** (12 pages). Ordering information is given on any current masthead page.

Kinetic Importance of Conformations of Nicotinamide Adenine Dinucleotide in the Reactions of Dehydrogenase Enzymes

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Suggestions regarding the relationship of cofactor conformation to stereospecificity[1] and to rates[2] of dehydrogenase enzymes have emerged. Our objectives have been to evaluate the potential energies of ground-state conformations and their influence on reaction trajectories and the structures of transition states. To assess the importance of conformational features, we have employed semiempirical (AM1)[3] and molecular dynamics ($CHARM_m$)[4] calculations using single-crystal X-ray structures of both nicotinamides and 1,4-dihydronicotinamides[5] and dehydrogenase enzymes.[6] The virtual angles X_n, X_{am}, α_C, and α_N define the conformations of interest (Charts I and II).

(1) (a) Benner, S. A. *Experentia* **1982**, *38*, 633. (b) Nambiar, K. P.; Stauffer, D. M.; Kolodzjiej, P. A.; Benner, S. A. *J. Am. Chem. Soc.* **1983**, *105*, 5886. (c) Benner, S. A. In *Mechanistic Principles of Enzymatic Activity*; Liebman, J. F., Greenberg, A., Eds.; VCH Publishers Inc.: New York, 1988; pp 56–59.

(2) (a) Cook, P. F.; Oppenheimer, N. J.; Cleland, W. W. *Biochemistry* **1981**, *28*, 1817. (b) Rotberg, N. S.; Cleland, W. W. *Biochemistry* **1991**, *30*, 4068.

(3) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902. AM1 was used from within MOPAC 6.0 (QCPE 455).

(4) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187. $CHARM_m$ v. 21.3 (Polygen-MSI) was implemented on a Silicon Graphics workstation, and Quanta 3.2 (Polygen-MSI) was used for structure visualization.

(5) Cambridge datafile, available from the Medical Foundation of Buffalo, Inc., Research Institute, 73 High Street, Buffalo, NY 14203-1196.

(6) The following X-ray structures were obtained from the Brookhaven database (July 1991 release, Brookhaven National Laboratory). (a) Dogfish lactate dehydrogenase ternary complex with oxamate (pdb file 1LDM): Abad-Zapatero, C.; Griffith, J. P.; Sussman, J. L.; Rossmann, M. G. *J. Mol. Biol.* **1987**, *198*, 445. (b) *L. casei* dihydrofolate reductase (pdb file 3DFR): Filman, D. J.; Bolin, J. T.; Matthews, D. A.; Kraut, J. *J. Biol. Chem.* **1982**, *257*, 13663. (c) Lobster glyceraldehyde 3-phosphate dehydrogenase (pdb file 1GPD): Moras, D.; Olsen, K. W.; Sabesan, M. N.; Buehner, M.; Ford, G. C.; Rossmann, M. G. *J. Biol. Chem.* **1975**, *250*, 9137. (d) Porcine heart malate dehydrogenase (pdb file 4MDH): Birktoft, J. J.; Rhodes, G.; Banacsak, L. J. *Biochemistry* **1989**, *28*, 6065.

(9) Sutherland, J. K.; Widdowson, P. A. *J. Chem. Soc.* **1964**, 4650.

(10) Prakash, C.; Sateh, S.; Blair, I. A. *Tetrahedron Lett.* **1989**, *30*, 19.

(11) An intermediate, presumed to be the C1,2 oxazoline, was observed by ^1H NMR spectroscopy prior to HPLC purification. This compound was spontaneously converted to the final product upon HPLC processing.

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Chart I

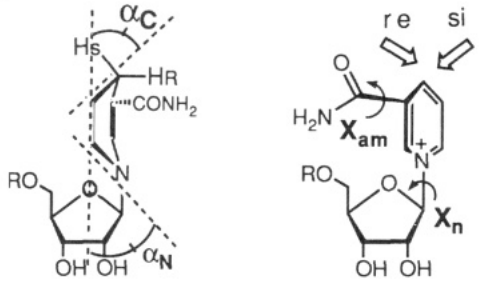
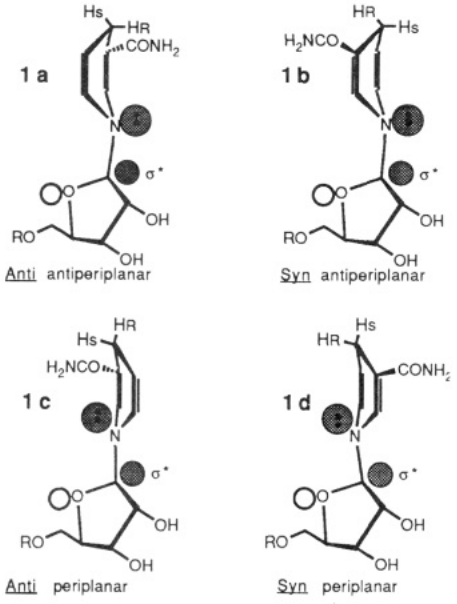


Chart II



According to Benner,[1] A-side (H_R) transfer involves the more exergonic reactions ($K_e = 10^{-11.3}$–$10^{-17.5}$), and B-side (H_S) transfer involves the less exergonic reactions ($K_e = 10^{-7.5}$–$10^{-11.2}$) (eq 1).

$$\text{NAD(P)H} + \text{S}_{ox} \overset{K_e}{\rightleftharpoons} \text{NAD(P)}^+ + \text{SH}_{red} \qquad (1)$$

He proposed that anti antiperiplanar NADH is a weaker reducing agent and is the conformation of the cofactor for A-side dehydrogenases, while the syn antiperiplanar NADH is the conformation of the cofactor in the B-side dehydrogenases (Chart II) such that all K_e values would become comparable by using the weaker reductant for the more exergonic reaction and the stronger reductant for the less favorable process.

Reaction trajectories were calculated using the placement of reactants as seen in the X-ray structure of dogfish lactate dehydrogenase[6a] using both anti antiperiplanar and syn antiperiplanar conformations of the cofactor. For these calculations initial values of X_n were chosen to reflect the most stable orientations for the two conformations and allowed to change during the course of reaction. The difference in the AM1 potential energies of the two transition states formed from the two conformations is less than 1 kcal/mol. This trivial difference is hardly sufficient to compensate for the great differences (up to 10^{10}) in K_e (eq 1). Thus, there is no basis for Benner's stereoelectronic hypothesis.[1]

Quasi-boat conformations (α_C and $\alpha_N > 0$) have been suggested to be of kinetic importance.[1,2] We have carried out molecular dynamics (MD)[4] simulation experiments employing CHARM$_m$[4] and the deposited[6] X-ray structures of dogfish muscle lactate dehydrogenase, D-glyceraldehyde-3-phosphate dehydrogenase, *L. casei* dihydrofolate reductase, and porcine heart malate dehydrogenase. For each enzyme, anisotropic quasi-boat puckering of the 1,4-dihydropyridine ring is observed. Average values of α_C and α_N equal +10° and +8°, respectively, for the unidirectional bending of the 1,4-dihydronicotinamide ring in dogfish lactate dehydrogenase. This unidirectional motion places the H_R in the pseudoaxial position directed toward the substrate (inset to Figure 1). Bending of the NADH dihydropyridine ring in a direction away from the substrate is hampered, by nonpolar amino acid side chains which form a "back wall" behind C4 of the dihydropyridine ring.[7] The kinetic importance of quasi-boat conformations was investigated using AM1 calculations. The results are shown in Figure 1 using $\alpha_N = 5°$ and $\alpha_C = 15°$. With the assumption that quasi-boat conformations are along the reaction path, the potential energy of the transition state is lowered by 6 kcal/mol. Formation of the quasi-boat conformation of NADH requires 1.8 kcal/mol, and for NAD^+ the requirement is 15 kcal/mol. Inspection of Figure 1 shows that the anisotropic motion brings the potential energies of quasi-boat NAD^+ and NADH ground states into comparison.

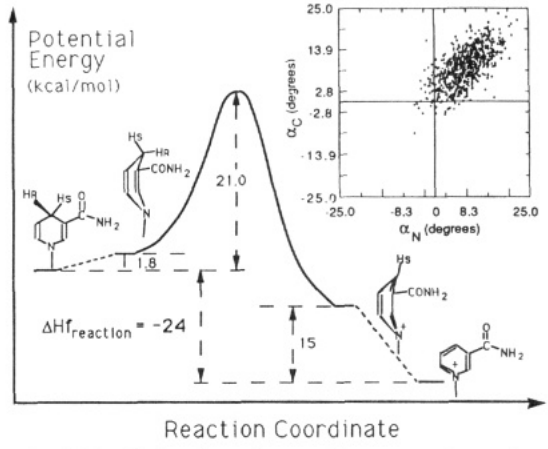
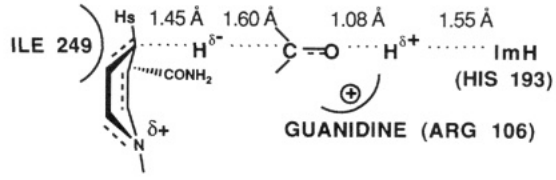


Figure 1. AM1 calculated reaction coordinate assuming anti antiperiplanar quasi-boat conformations for both NAD(P)H and $NAD(P)^+$ ($\alpha_N = 5°$ and $\alpha_C = 15°$) along the reaction coordinate. Puckering is in the direction of the substrate formaldehyde. Inset: Plot of α_C and α_N from the 40-ps collection phase of MD simulation with dogfish muscle lactate dehydrogenase active ternary complex with pyruvate (see refs 4 and 6a). α_C is positive in over 97% of the structures (800 total) showing that puckering to a quasi-boat conformation of NADH occurs exclusively in the direction of the substrate. The X-ray structure of dogfish muscle lactate dehydrogenase (see ref 6a) has been used as the basis for all calculations. For the MD computations, pyruvate substrate was superimposed on the oxamate pseudosubstrate of the X-ray structure and the imidazole of HIS 193 was protonated. For AM1 calculations, the guanido group of methylguanidine was superimposed on the guanidino group of ARG 106, a protonated imidazole was superimposed on the imidazole group of HIS 193, the carbonyl of the substrate (formaldehyde) was overlapped with the *pseudo*substrate oxamate amide carbonyl, the dihydropyridine moiety of the appropriately chosen conformation of $N(\beta,1)$-ribosyl 1,4-dihydronicotinamide was placed such that the mean plane of atoms 2, 3, 5, and 6 of the nicotinamide ring overlapped with the corresponding atoms of NADH in the X-ray structure (a torsion angle $X_{am} = 150°$ was used based on the structures of the dehydrogenases of interest).

Chart III



Using the X-ray structure of the dogfish enzyme,[6] with the cofactor in anti antiperiplanar conformation ($\alpha_N = 5°$ and $\alpha_C = 15°$), the reaction trajectory was calculated by AM1. The computed trajectory suggests a very late transition state (Chart III) for general-acid proton transfer to substrate oxygen ($-\alpha \approx 0.9$) and a midway transition state for hydride transfer to carbon. Such

(7) The hydrophobic residue side chains forming the "back wall" in the respective dehydrogenase structures are (a) Ile 249 in dogfish lactate dehydrogenase, (b) Phe 103 in *L. casei* dihydrofolate reductase, (c) Ile 12 in lobster glyceraldehyde 3-phosphate dehydrogenases, and (d) Ala 245 and Leu 157 in porcine heart malate dehydrogenase.

a mechanism has much of the advantage of specific-acid catalysis but retains the essential feature of general-acid catalysis of being able to localize the proton. Complete proton transfer in the transition state has the advantage of full polarization of the carbonyl bond.[8] These results are much like the recent proposal[9] of late transition states for protonation in the concerted enzymatic general-acid–general-base catalysis of carbon acid enolization.

Acknowledgment. This work was supported by grants from the National Science Foundation and Office of Naval Research.

Registry No. NADH, 58-68-4; NAD^+, 53-84-9; D-glyceraldehyde-3-phosphate dehydrogenase, 9028-92-6; lactate dehydrogenase, 9001-60-9; dihydrofolate reductase, 9002-03-3.

(8) Resonance Raman studies on lactate dehydrogenase with pyruvate covalently bound to NAD^+ indicate a large degree of polarization of the carbonyl: Deng, H.; Zheng, J.; Burgner, J.; Callender, R. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 4484.

(9) Gerlt, J. A.; Kozarich, J. W.; Kenyon, G. L.; Gassman, P. G. *J. Am. Chem. Soc.* **1991**, *113*, 9667.

Synthetic Sapphyrin–Cytosine Conjugates: Carriers for Selective Nucleotide Transport at Neutral pH

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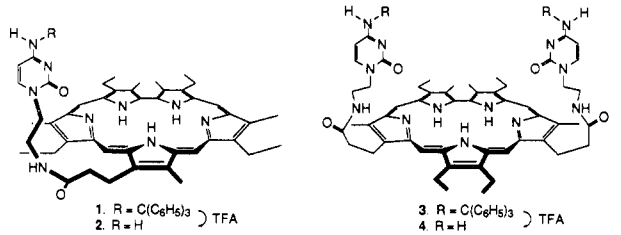
Anionic phosphorylated entities are ubiquitous in biology. They play a critical role in a variety of fundamental processes ranging from gene replication to energy transduction.[1] In addition, certain phosphate-bearing nucleotide analogues, such as, for example, 9-(β-D-*xylo*-furanosyl)guanine 5′-monophosphate (Xylo-GMP), are known to display antiviral activity in vitro.[2] Not surprisingly, therefore, in recent years, increasing effort has been devoted to the problem of phosphate recognition, and a number of elegant phosphate-binding receptors are now known.[3] In spite of this, we are unaware of any artificial entity that is capable of effecting the selective through-membrane transport of guanosine-derived mononucleotides (e.g., guanosine 5′-monophosphate, GMP) at neutral pH or making organic soluble these normally organic insoluble entities. We now wish to report the synthesis of a new cytosine–sapphyrin conjugate, **2**, that acts as a selective carrier for the through-membrane transport of GMP at neutral pH in an Aq I–CH_2Cl_2–Aq II (Aq = aqueous) model membrane system. We also wish to report the preparation of a related doubly substituted analogue **4**.[4]



In prior work, we reported that organic-solubilized, 2′,3′,5′-tris(triisopropylsilyl)-substituted nucleosides would enhance the through-CH_2Cl_2 transport of the corresponding Watson–Crick complementary phosphate-free nucleoside in a standard three-phase Aq I–CH_2Cl_2–Aq II liquid membrane cell.[5] We also reported that the diprotonated form of sapphyrin, a pentapyrrolic "expanded porphyrin",[6] acts as an efficient but nonselective carrier for nucleotide monophosphates at pH < 4.[7] More recently,[8] we have found that a combination of rubyrin, a hexapyrrolic homologue of sapphyrin that is more difficult to prepare,[9] and 2′,3′,5′-tris(triisopropylsilyl)-substituted cytidine (C-Tips) in large (ca. 100-fold) excess was able to effect the selective through-transport of GMP at neutral pH. However, sapphyrin, which remains monoprotonated in the ca. 3.5 ≤ pH ≤ 10 regime,[7,8] was itself found to be ineffective as a GMP carrier at pH 7, even in the presence of a large excess of C-Tips.[8] Thus, it was thought that if sapphyrin-based systems were to be made effective as neutral regime carriers for GMP, it would require the construction of polytopic receptor systems, such as **2** and **4**, in which cytosine-like recognition units are "appended" directly onto the phosphate-chelating expanded porphyrin core.

Receptors **2** and **4** were prepared by trifluoroacetic acid (TFA) induced detritylation of the protected conjugates **1** and **3**. These, in turn, were prepared by coupling 1-(2-aminoethyl)-4-[(triphenylmethyl)amino]pyrimidin-2-one[10] with the appropriate sapphyrin mono- or diacid chlorides.[11] Transport studies were then carried out using a standard[12] Aq I–CH_2Cl_2–Aq II liquid membrane cell.[13]

As can be seen from Table I, both **2** and **4** are able to effect the selective through-membrane transport of GMP at, or near, neutral pH.[14] Interestingly, in all cases, receptor **2** displays a higher selectivity for GMP (by a factor of 8–100 relative to either

(1) *The Biochemistry of the Nucleic Acids*, 10th ed., Adams, R. L. P., Knowler, J. T., Leader, D. P., Eds., Chapman and Hall: New York, 1986.

(2) Holý, A. In *Approaches to Antiviral Agents*; Harden, M. R., Ed.; VCH Publishers: Deerfield Beach, FL, 1985; pp 101–134.

(3) For representative examples of earlier work in the phosphate recognition and/or transport area, see: (a) Tabushi, I.; Kobuke, Y.; Imuta, J. *J. Am. Chem. Soc.* **1981**, *103*, 6152–6157. (b) Kimura, E. *Top. Curr. Chem.* **1985**, *128*, 113–141. (c) Schmidtchen, F. P. *Top. Curr. Chem.* **1986**, *132*, 101–133. (d) Lehn, J.-M. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 89–112. (e) Marecek, J. F.; Fischer, P. A.; Burrows, C. J. *Tetrahedron Lett.* **1988**, *29*, 6231–6234. (f) Schmidtchen, F. P. *Tetrahedron Lett.* **1989**, *30*, 4493–4496. (g) Mertes, M. P.; Mertes, K. B. *Acc. Chem. Res.* **1990**, *23*, 413–418. (h) Hosseini, W.; Blacker, A. J.; Lehn, J.-M. *J. Am. Chem. Soc.* **1990**, *112*, 3896–3904. (i) Kimura, E.; Kuramoto, Y.; Koike, T.; Fujioka, H.; Kodama, M. *J. Org. Chem.* **1990**, *55*, 42–46. (j) Aoyama, Y.; Nonaka, S.; Motomura, T.; Toi, H.; Ogoshi, H. *Chem. Lett.* **1991**, 1241–1244. (k) Claude, S.; Lehn, J.-M.; Schmidt, F.; Vigneron, J.-P. *J. Chem. Soc., Chem. Commun.* **1991**, 1182–1185. (l) Deslongchamps, G.; Galán, A.; de Mendoza, J.; Rebek, J., Jr. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 61–63. (m) Dixon, R. P.; Geib, S. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **1992**, *114*, 365–366. (n) Ariga, K.; Anslyn, E. V. *J. Org. Chem.* **1992**, *57*, 417–419.

(4) Compounds **1–4** appear to be the first examples of nucleic acid derivatives ("nucleobases") conjugated to expanded porphyrins. Such conjugates, however, are known in the porphyrin series; see: (a) Sessler, J. L.; Magda, D. In *Inclusion Phenomena and Molecular Recognition*; Atwood, J., Ed.; Plenum Press: New York, 1990; pp 17–26. (b) Kus, P.; Knerr, G.; Czuchajowski, L. *Tetrahedron Lett.* **1990**, *36*, 5133–5134. (c) Hisatome, M.; Maruyama, N.; Furutera, T.; Ishikawa, T.; Yamakawa, K. *Chem. Lett.* **1990**, 2251–2254. (d) Harriman, A.; Kubo, Y.; Sessler, J. L. *J. Am. Chem. Soc.* **1992**, *114*, 388–390.

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(6) (a) Bauer, V. J.; Clive, D. L. J.; Dolphin, D.; Paine, J. B., III; Harris, F. L.; King, M. M.; Loder, H.; Wang, S.-W.C.; Woodward, R. B. *J. Am. Chem. Soc.* **1983**, *105*, 6429–6436. (b) Broadhurst, M. J.; Grigg, R.; Johnson, A. W. *J. Chem. Soc., Perkin Trans. 1* **1972**, 2111–2116. (c) Sessler, J. L.; Cyr, M. J.; Lynch, V.; McGhee, E.; Ibers, J. A. *J. Am. Chem. Soc.* **1990**, *112*, 2810–2813. (d) Sessler, J. L.; Cyr, M. J.; Burrell, A. K. *Synlett* **1991**, 127–133.

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(10) Sessler, J. L.; Magda, D.; Furuta, H. *J. Org. Chem.* **1992**, *57*, 818–826.

(11) Satisfactory spectroscopic and analytical data was obtained for all new compounds; see supplementary material.

(12) Tsukube, H. In *Liquid Membranes: Chemical Applications*; Araki, T., Tsukube, H., Eds.; CRC Press: Boca Raton, FL, 1990; pp 27–50.

(13) Transport experiments were performed in a manner similar to that detailed in refs 5 and 7.

(14) Using our standard (ref 7) detection system, a quantitative analysis of uridine 5′-monophosphate (UMP) in Aq II could not be made. It has, therefore, been excluded from the present study. In qualitative control experiments, it was confirmed that carrier **2** caused no apparent increase in the rate of through-membrane UMP transport at pH 6.7.

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