Protonated Sapphyrins. Highly Effective Phosphate Receptors

Vladimír Král, Hiroyuki Furuta, Kevin Shreder, Vincent Lynch, and Jonathan L. Sessler*

Contribution from the Department of Chemistry and Biochemistry, The University of Texas at Austin, Austin, Texas 78712-1167

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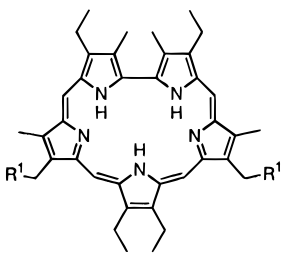
Abstract: The phosphate anion chelation properties of several sapphyrin derivatives, namely 3,12,13,22-tetraethyl-8,17-bis[bis(hydroxyethyl)amino)carbonylethyl]-2,7,18,23-tetramethylsapphyrin (**1**), 3,12,13,22-tetraethyl-2,7,18,23-tetramethyl-8,17-bis(hydroxypropyl)sapphyrin (**2**), and 3,8,12,13,17,22-hexaethyl-2,7,18,23-tetramethylsapphyrin (**3**) are reported. X-ray diffraction studies of the dihydroxylated and decaalkyl derivatives **2** and **3** reveal that the diprotonated forms of sapphyrin are capable of stabilizing 1:2 inner-sphere complexes with phosphate-derived monoanions, such as diphenyl phosphate and monobasic phenyl phosphate. Similar analyses reveal that the diprotonated form of dihydroxysapphyrin **2** is capable of forming a 1:1 chelate complex in the solid state with either mono- or dibasic phosphoric acid. Solution-phase studies, involving ^{1}H and ^{31}P NMR spectroscopy, confirm that these same sapphyrins are capable of binding phosphate anions in organic solution, a conclusion that is supported by qualitative fast atom bombardment mass spectrometric (FAB MS) and extractive partition studies. In the case of phenylphosphonic acid and sapphyrin **2**, extraction studies were consistent with 2:1 and 1:1 phosphate-to-sapphyrin binding stochiometries at pH 1.68 and 5.6, respectively. Similar studies using NMR and visible spectroscopy carried out with the water-soluble tetrahydroxy sapphyrin derivative, **1**, and **2** indicate that these species bind phosphate anion in both methanolic and aqueous solution. Calculated association constants are on the order of 10^4 M^{-1} in methanol and 10^2 M^{-1} in 10 mM aqueous bis-Tris, pH 6.1.

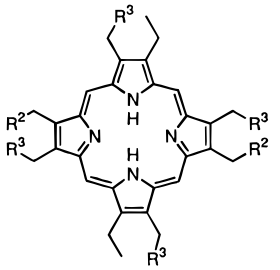
Introduction

Phosphate anions and their derivatives are ubiquitous in biology. They play a key role in a wealth of critical processes ranging from information processing[1] to energy storage and transduction.[2] They are also present in the active, phosphorylated forms of many antiviral agents.[3] Not surprisingly, therefore, considerable effort has been devoted recently to the development of phosphate anion receptors and carriers. At present, however, only a few such synthetic phosphate binding motifs are known.[4] These include quarternary amines,[4a−c] polyamines and cyclic polyamines,[4d−q] guanidinium and guanidinium-like receptors,[4r−ag] metalloreceptors[4ah−al] (of, e.g., the UO_2−salene type), and multiple hydrogen bonding receptors[4am−aq] with well-defined geometries (based on, e.g., calixarene and cyclodextrin frameworks). Only for a fraction of these is confirmatory solid-state X-ray crystallographic information available.[4aa,ad,af,aj,aq] This stands in contrast to the generalized problem of receptor-based anion recognition, for which a vast body of both solution-phase and solid-state binding information is now available.[5] In this paper, therefore, we wish to present what we believe is a unique approach to phosphate anion chelation. It is one that is based on the use of protonated sapphyrins,[6] a specific pentapyrrolic subset of what is a general class of large porphyrin-like macrocycles known as expanded porphyrins.[7]

The sapphyrins (e.g. **1**−**3**), first reported by Woodward some 30 years ago,[8] bear considerable resemblance to their far better-studied porphyrin analogues (e.g. **4** and **5**). They differ, however, in a number of ways. They contain, for instance, five, rather than four, pyrrolic subunits. In addition, the sapphyrins are best characterized as being 22, rather than 18, π-electron aromatic systems and are green, rather than purple, in color. The sapphyrins are also substantially larger than the porphyrins, possessing, for instance, an inner cavity of ca. 2.5 Å radius (making them roughly 25% bigger than the porphyrins, for which cavity radii on the order of 2.0 Å are generally observed[9]). As evidenced by solid-state X-ray diffraction analyses, the larger core size of sapphyrin allows the diprotonated form of this macrocycle to remain planar without any significant distortion caused by the Coulombic and Van der Waals interactions of the protons bound within its core. By contrast, the diprotonated forms of porphyrins are known to be highly distorted in the solid state.[9a,b] In part because of this larger core size, sapphyrins are also far more basic than the porphyrins, remaining effectively monoprotonated at neutral pH and doubly protonated at or below ca. pH 3.5 (for sapphyrin **1**, $pK_{a1} = 4.8$, $pK_{a2} = 8.8$; *vide infra*). Typical, unsubstituted porphyrins, on the other hand, are unprotonated at neutral pH and are effectively monoprotonated only at quite low pH.[10]



1 $R^1 = CH_2CON(CH_2CH_2OH)_2$
2 $R^1 = CH_2CH_2OH$
3 $R^1 = CH_3$



4 $R^2 = CON(CH_2CH_2OH)_2$, $R^3 = H$
5 $R^2 = R^3 = CH_3$

For some time we have been interested in developing further

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the chemistry of the sapphyrins,[6,11] as well as that of other expanded porphyrins.[12] In the course of this work, we have recently discovered a unique feature of certain expanded porphyrins, namely that they act as halide anion receptors in their protonated forms.[13] Initial evidence that the sapphyrin macrocycle was capable of halide binding was derived from single-crystal X-ray diffraction analyses. For example, in early work, it was found that in the fluoride complex $[[2H\cdot\mathbf{3}]^{2+}\cdot F^{-}]^{+}$, the fluoride counter anion is encapsulated within the sapphyrin core *via* a combination of simple Coulombic and H-bonding interactions.[6a] Later solution studies confirmed this binding motif and found a high degree of selectivity for fluoride anion over both chloride and bromide.[12,13]

This halide anion binding behavior, which stands in marked contrast to that of the simpler porphyrins, led us to consider that sapphyrins might be capable of acting as receptors for other biologically important anions, including phosphate. In previous work involving U-tube type model membrane transport studies, it was found that diprotonated sapphyrins can act as solution-phase carriers for nucleotides[14] and nucleotide analogues[15,16ab] and provided a qualitative indication that sapphyrins can bind or chelate phosphate anions in organic solvents. We also found that a silica-bound sapphyrin system provided a useful solid support for the HPLC separation of monomeric and short oligomeric nucleotides at pH 7[16c] and, as detailed in the ensuing paper, found that certain water-soluble sapphyrins (e.g., **1**) bind DNA in aqueous pH 7 solution.[17ab]

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In the present report, we wish to provide more quantitative support for the idea that protonated sapphyrins can act as highly effective phosphate anion receptors. In particular, we wish to report the results of X-ray diffraction studies showing that the diprotonated forms of sapphyrins **2** and **3** are capable of stabilizing 1:2 complexes with diphenyl phosphate and monobasic phenyl phosphate, respectively, and that the diprotonated form of sapphyrin **2** can bind either mono- or dibasic phosphate as a 1:1 chelate complex in the solid state. We also present the results of ^{1}H and ^{31}P NMR solution-phase studies that, along with liquid−liquid extraction and FAB MS analyses, support the conclusion that these two sapphyrins act as phosphate anion receptors in both methanol and water solutions. Finally, we present the synthesis of the water-soluble tetrahydroxysapphyrin derivative **1** and show that it acts as a phosphate anion chelating agent in aqueous solution.[17] None of this chemistry, either in terms of the present solid-state and solution-phase phosphate anion recognition or previously reported nucleotide transport,[15,16a,b] is reproduced in the case of the corresponding porphyrins (e.g. **4** and **5**).

Experimental Section

General Methods. ^{1}H NMR spectra were recorded on General Electric QE-300 (300 MHz) and GE GN500 instruments. ^{31}P NMR spectra were recorded on Nicolet NT-360 (81 MHz) and Bruker AM500 spectrometers with phosphoric acid being used as an external reference. UV−vis spectra were recorded on a Beckman DU 640 instrument using cuvettes of either 1 cm or 1 mm path length. Samples of high optical density were studied as ca. 0.1-mm films.

Purification of Sapphyrin and Porphyrin Macrocycles. Free-base sapphyrins for NMR titration experiments were prepared from the corresponding diprotonated forms (as the bis-chloride or bis-tosylate salts) as follows: A small sample of the sapphyrin salt (10 to 50 mg) was dissolved in 50 mL of dichloromethane and then washed with 20 mL of 1 M NaOH in a separatory funnel. The organic layer was then separated off and dried over sodium sulfate. A final chromatographic purification on neutral alumina was performed using dichloromethane/methanol (2−10% v/v) as the mobile phase. Sapphyrin and porphyrin samples used for NMR studies were thoroughly dried *in vacuo* and then stored under argon at −15 °C prior to use. The original diprotonated sapphyrin salts were isolated by column chromatography on silica gel using dichloromethane/methanol (1−10% v/v) and then purified further by recrystallization from dichloromethane/pentane. The monohydrochloride salt of sapphyrin **2** was prepared from the free-base form by washing with dilute hydrochloric acid (pH 5) and drying over magnesium sulfate. The corresponding bishydrochloride salt, **2**·2HCl, was prepared by washing with 1 M HCl and drying over magnesium sulfate. Final purification of sapphyrin **1** and porphyrin **4** was made *via* reverse-phase column chromatography using methanol as the eluent. Phenylphosphonic acid, diphenyl phosphate, and phenyl phosphate disodium salt were obtained from commercial suppliers (Aldrich and/or Sigma Chemical companies).

Binding Studies. The association constants were determined from both ^{1}H NMR and ^{31}P NMR spectroscopic titrations carried out in methanol-d_4 and visible spectroscopic studies carried out in both methanol and aqueous bis-Tris buffer. For the ^{1}H and ^{31}P NMR studies, the concentration of the phosphorylated species was kept constant (0.5−5 mM) and the change in the chemical shift was followed as a function of increasing macrocycle concentration. For the visible spectroscopic studies, the concentration of macrocycle was kept constant and the change in absorbance at a given wavelength was followed as the concentration of the phosphorylated species in question was increased. For the NMR and vis studies carried out in organic solvents (e.g., methanol), data reduction was performed using standard methods[18] in accord with specific procedures outlined earlier.[4z,aa,ab,13,16] For the vis titrations carried out in aqueous media, bis-Tris buffer (10 mM, pH 6.1) was used. The concentration of the water-soluble sapphyrin **1** was kept constant at 6 μM while increasing quantities (0−2000 equiv) of the phosphate species (e.g., phosphoric acid, phenyl phosphate, phenylphosphonate) in question were titrated in. Apparent association constants were determined using iterative, curve-fitting procedures (KaleidaGraph, version 3.0.2). After entering initial guesses for the association constant (K_a) and the difference in the A_{420} value of the dimeric sapphyrin-bound phosphate complex and free sapphyrin (A_c), these variables were allowed to float freely over the iteration. Association constants derived from UV−visible titration data were calculated from a plot of the observed change in the absorbance at 420 nm (A) of **1** as a function of the concentration of added phenyl phosphate or phenylphosphonate (C) according to the following formula:

$$A = \frac{[(K_aC + K_aR + 1) - \sqrt{(K_aC + K_aR + 1)^2 - 4K_a^2CR}]A_c}{2K_aR}$$

wherein R represents the initial, fixed concentration of sapphyrin **1** (generally 6×10^{-6} M).

For the studies carried out in organic media, it was generally ascertained (cf. Results and Discussion) that two types of complexes were being formed, namely a weaker 1:2 sapphyrin-to-phosphate complex and a much stronger 1:1 complex as based on the shape of the observed titration curves (shift versus sapphyrin/phosphate molar ratio). As a general rule, it was found that experimental conditions could be found (e.g., macrocycle **2**, phosphate and sapphyrin concentrations both at ≤3 mM concentrations in methanol) where various putative phosphate- or sapphyrin-derived self-aggregation processes,[4ab,ae] manifested by deviations from Beer's law behavior (UV-vis studies) or poor fits to clean 1:1 and 1:2 binding profiles in the NMR analyses, could be safely ignored. However, this was not always the case (see Results and Discussion). In these instances, quantitative conclusions were necessarily precluded.

Extraction Studies. Solutions of phenylphosphonic acid (20 mL at 0.05 M), at either pH 1.68 (free-acid) or 5.60 (sodium salt, generated *in situ* by addition of NaOH), were stirred for 20 h in the presence of 1 mM dichloromethane solutions of the free-base sapphyrins **2** or **3** or control porphyrin **5** (20 mL volume). The organic phase was separated, dried over sodium sulfate, and evaporated to dryness. The ratio of sapphyrin-to-phosphonate anion present in the resulting material was then determined by integration of the ^{1}H NMR spectra (comparing the integrated intensity of the sapphyrin methine protons to those of phenylphosphonate) recorded in chloroform-*d* containing 5% by volume methanol-d_4. The same results were obtained when phenyl phosphate (as the free-acid for studies at pH 1.6 and as the sodium salt, generated *in situ* by addition of NaOH, for studies at pH 5.6) was used in the place of phenylphosphonate. In other words, a 1:2 sapphyrin-to-phosphate complex was observed at pH 1.6 and a 1:1 complex was found at pH 5.6. Control extraction experiments with octaethylporphyrin resulted in no extraction of these or other phosphorylated species at pH 5.6. In addition, no MS evidence was obtained that could be considered as being consistent with phosphate binding (i.e., complex formation) in the case of porphyrins **4** and **5**.

X-ray Structural Analyses. Single crystals of sapphyrin salts **3**·3[$C_6H_5OPO(OH)_2$] and **2**·2[$(C_6H_5O)_2PO(OH)$], corresponding to the neutral 1:2 first coordination sphere complexes $[2H\cdot\mathbf{3}]^{2+}\cdot 2[C_6H_5OP(O)(OH)O]^-$ and $[2H\cdot\mathbf{2}]^{2+}\cdot 2[(C_6H_5O)_2P(O)O]^-$, respectively, were obtained by vapor diffusion of diethyl ether into dichloromethane/methanol (1:1 v/v) solutions of the appropriate complex salts. Two sets of phosphoric acid-derived crystals, **2**·3[H_3PO_4]·0.68H_2O and **2**·[H_3PO_4]·H_2O, corresponding to the charged and neutral 1:1 inner-sphere complexes $[[2H\cdot\mathbf{2}]^{2+}\cdot[H_2PO_4]^-]^+$ and $[2H\cdot\mathbf{2}]^{2+}\cdot[HPO_4]^{2-}$, respectively, were studied. These were prepared by dissolving sapphyrin **2** in chloroform/methanol (10:1 v/v) and washing with phosphoric acid at pH 1.9. The organic layer was separated off and dried over $MgSO_4$. It was then redissolved in dichloromethane/methanol (9:1 v/v) with

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Table 1. Crystallographic Data[a] for Sapphyrin Salts **3**·3[$C_6H_5OPO(OH)_2$] (**I**), **2**·3[H_3PO_4]·0.68H_2O (**II**), **2**·2[$(C_6H_5O)_2PO(OH)$] (**III**), and **2**·[H_3PO_4]·H_2O (**IV**)

	I	**II**	**III**	**IV**[f]
formula	$C_{58}H_{70}N_5P_3O_{12}$	$C_{42}H_{62}N_5O_{14}P_3$·0.68H_2O	$C_{64}H_{71}N_5P_2O_8$	$C_{42}H_{58}N_5PO_7$
fw	1122.14	966.17	1100.24	775.90
a, Å	11.644(3)	11.518(4)	13.868(2)	10.497(5)
c, Å	17.594(6)	17.099(7)	17.352(2)	15.189(7)
b, Å	14.624(3)	13.545(4)	14.6998(14)	12.928(5)
α, deg	90.90(2)	68.19(3)	66.057(9)	82.96(4)
β, deg	102.60(2)	75.06(3)	80.188(10)	81.13(4)
γ, deg	97.99(2)	79.67(3)	63.054(8)	85.46(3)
V, Å^3	2892.1(14)	2383(2)	2881.6(6)	2017(2)
Z	2	2	2	2
F(000)	1188	1025.6	1168	832
crystal system	triclinic	triclinic	triclinic	triclinic
space group	$P\bar{1}$ (No. 2)	$P\bar{1}$	$P\bar{1}$	$P\bar{1}$
T, °C	25	25	25	−100
2θ range, deg	4−45	4−45	4−45	4−45
scan/speed, deg/min	2−5	2−5	4.1−5.5	4−8
ρ_{calc}, g/cm^3	1.29	1.35	1.27	1.28
no. of reflcns measured	8328	6949	10101	5630
no. of unique reflcns	7614	6263	7505	5284
R_{int}	0.032	0.020	0.020	0.043
μ, cm^{-1}	1.61	1.88	1.30	1.24
transmission factor[b] range	0.967→0.979	0.982→0.991	not applied	not applied
crystal size, mm	0.14 × 0.21 × 0.21	0.06 × 0.08 × 0.70	0.08 × 0.26 × 0.33	0.10 × 0.10 × 0.87
no. of reflcns used	3392	3430	2573	5284
no. of reflcns rejected	4222 [$F < 4(\sigma(F))$]	2833 [$F < 4(\sigma(F))$]	4932 [$F < 4(\sigma(F))$]	0
R(F)[c,d]	0.0995	0.0784	0.104	0.070
$R_w(F)$	0.0945	0.0759	0.0859	0.183
goodness of fit[e]	1.932	1.863	1.682	1.076
no. of parameters	577	594	324	524
max $\lvert\Delta/\sigma\rvert$	0.12	<0.1	<0.1	0.12
min, max peaks (e/Å^3)	−0.57, 0.74	−0.40, 0.48	−0.47, 0.72	−0.38, 0.64

[a] Data for **I** and **II** were collected at room temperature and data for **IV** were collected at −100 °C on a Nicolet P3 diffractometer. Data for **III** were collected at room temperature on an Enraf-Nonius CAD4 diffractometer. All data were collected using graphite monochromatized Mo Kα radiation, $\lambda = 0.71073$ Å. Lattice parameters were obtained from the least-squares refinement of 29 reflections with $10.1 < 2\theta < 17.9°$ for **I**, 36 reflections with $10.8 < 2\theta < 18.6°$ for **II**, 23 reflections with $8.2 < 2\theta < 20.7$ for **III**, and 21 reflections with $8.7 < 2\theta < 19.3°$ for **IV**. [b] Absorption correction was based on measured crystal faces. [c] The function $\sum w(|F_o| - |F_c|)^2$ was minimized where $w = 1/(\sigma(F_o)^2 + (0.02F)^2)$. [d] $R(F) = \sum(|F_o| - |F_c|)/\sum(|F_o|)$. $R_w(F) = \{\sum w(|F_o|^2 - |F_c|)^2/\sum w|F_o|^2\}^{1/2}$. [e] $S = [\sum w(|F_o| - |F_c|)^2/(n - p)]^{1/2}$, where n is the number of reflections and p is the number of refined paramters. [f] The following definitions apply to **IV** which was refined on F^2. $R_w = \{\sum w(|F_o|^2 - |F_c|)^2)^2/\sum w(|F_o|^4\}^{1/2}$, where the weight, w, is defined as follows: $w = 1/\{\sigma^2(|F_o|^2) + (a*P)^2 + b*P\}$; $P = [1/3*(\text{maximum of } (0 \text{ or } |F_o|^2) + 2/3*|F_c|^2]$. The parameters a and b were suggested during refinement and are 0.0687 and 2.971, respectively. The conventional R index based on F where the 3485 observed reflections have $F_o > 4(\sigma(F_o))$. $S = [\sum w(|F_o|^2 - |F_c|)^2)^2/(n - p)]^{1/2}$, where n is the number of reflections and p is the number of refined parameters.

single crystals being obtained by slow vapor diffusion of diethyl ether into this solution. This gave crystals with empirical composition **2**·[H_3PO_4]$_3$·0.68H_2O. Crystals with composition **2**·[H_3PO_4]·H_2O were obtained by extraction at pH 6.0 followed by an identical crystallization procedure. Data for **3**·3[$C_6H_5OPO(OH)_2$] and **2**·3[H_3PO_4]·0.68H_2O were collected at room temperature on a Nicolet P3 diffractometer while data for **2**·[H_3PO_4]·H_2O were collected at −100 °C on this same instrument. Data for **2**·2[$(C_6H_5O)_2PO(OH)$] were collected at room temperature on an Enraf-Nonius CAD4 diffractometer. All data were collected using graphite monochromatized Mo Kα radiation, $\lambda = 0.71073$ Å. Details of crystals are listed in Table 1 with selected bond lengths and angles being provided in the captions to Figures 1−6. Other pertinent information, including X-ray experimental details for each complex, is included in the supporting information.

pK_a Titration. An aqueous 2.5% Tween 20 (Aldrich Chemical Company) solution of the bishydrochloride salt of sapphyrin **1** (1.0×10^{-3} M) was prepared with argon-bubbled, doubly deionized water (Barnstead, NANOpure II) and titrated with a carbonate free aqueous solution of 0.1 M NaOH (Aldrich Chemical Company) at 25 °C under a steady stream of humidified Ar. pH values were read with an Orion 720 digital pH meter calibrated with pH 4.00 and 7.00 standard buffer solutions. The resulting data points were plotted as pH versus equivalents of NaOH added and a first derivative of the smoothed data points was taken. The resulting minima were used to determine the pK_{a1} and pK_{a2} of sapphyrin **1**.

Syntheses. 3,8,12,13,17,22-Hexaethyl-2,7,18,23-tetramethylsapphyrin (**3**) was prepared as previously described.[6a,10] Octaethylporphyrin (**5**) was made according to a published literature procedure.[19]

1,12,13,22-Tetraethyl-8,17-bis[bis(hydroxyethyl)amino)-carbonylethyl]$^-$2,7,18,23-tetramethylsapphyrin (1). 3,12,13,22-Tetraethyl-8,17-bis(carboxyethyl)-2,7,18,23-tetramethylsapphyrin[16] (69 mg, 0.1 mmol) was partially dissolved in dry dichloromethane (30 mL) and to this suspension was added 0.5 mL of oxalyl chloride along with 1 drop of DMF under argon. The resulting reaction mixture was stirred at room temperature for 3 h and then evaporated to dryness. The sapphyrin bisacid chloride so produced was redissolved in dry dichloromethane (20 mL) and slowly added under argon to a solution of diethanolamine (52.5 mg, 0.5 mmol) in dry dichloromethane (30 mL) or tetrahydrofuran (30 mL), which also contained a catalytic amount (ca. 5 mg) of 4-(dimethylamino)pyridine and 0.2 mL of pyridine. The reaction mixture was stirred at room temperature for 24 h and then evaporated to dryness. A solution of 1 M NaOH (30 mL) was added and the resulting suspension stirred for an additional 10 min. The precipitated product was then filtered off, washed with cold water (30 mL), and dried *in vacuo* before recrystallizing from ethanol/hexane (1:3). The free-base sapphyrin so obtained was not soluble in water; however, conversion to the readily water-soluble hydrochloric acid salt could be effected by adding 3 mL of 5% HCl and evaporating to dryness. Recrystallization from ethanol/hexane (1:3) then gave 75 mg (80%) of the bishydrochloride adduct of **1**. For **1**, ^{1}H NMR (300 MHz, $CDCl_3$): δ -5.13 (2H, s, NH), −4.95 (1H, s, NH), −4.78 (2H, s, NH), 2.00 (6H, t, CH_2CH_3), 2.12 (6H, t, CH_2CH_3), 3.26 (8H, m, NCH_2CH_2-OH), 3.48 (4H, t, CH_2CH_2CON), 3.85 (8H, m, NCH_2CH_2OH), 4.02 (6H, s, CH_3), 4.18 (6H, s, CH_3), 4.47 (4H, q, CH_2CH_3), 69 (4H, q, CH_2CH_3), 5.02 (4H, t, CH_2CH_2CON), 11.58 (2H, s, meso-H), 11.61 (2H, s, meso-H). ^{1}H NMR of **1** free-base (300 MHz, $CDCl_3$ with 10% CD_3OD): δ 1.98 (6H, t, CH_2CH_3), 2.11 (6H, t, CH_2CH_3), 3.13 (4H, t, $CONCH_2CH_2$), 3.42 (4H, t, CH_2CH_2CON), 3.59 (8H, t, CH_2CH_2OH),

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3.91 (6H, s, CH_3), 4.09 6H, s, CH_3), 4.39 (4H, q, C**H**$_2$CH$_3$), 4.57 (4H, s, C**H**$_2$CH$_3$), 4.94 (4H, t, C**H**$_2$CH$_2$CON), 11.16 (2H, s, methine), 11.18 (2H, s, methine). ^{13}C NMR (75 MHz, $CDCl_3$ with 10% CD_3OD): δ 12.46, 15.59, 16.02, 16.51, 17.76, 18.46, 20.52, 20.56, 23.23, 35.50, 36.39, 37.02, 51.63, 59.63, 60.67, 60.74, 75.66, 90.27, 90.50, 90.91, 97.94, 127.17, 130.88, 135.74, 136.07, 137.14, 137.53, 142.55, 142.69, 172.98. FAB MS *m/e* (rel intensity) 862 (98, $[M + H]^+$), 863 (78, $[M + 2H]^+$), 861 (56, $[M]^+$). HRMS Calcd for $C_{50}H_{68}N_7O_6$ 862.520676, obsd 862.523102. UV−vis (H_2O, pH 7.0): λ_{max} (ϵ) 400 (98 000), 674 (4 200), 623 (5 600) Anal. Calcd for $C_{50}H_{67}N_7O_6\cdot H_2O$: C, 68.23; H, 7.83; N, 11.17. Found: C, 68.11; H, 7.85; N, 11.04. Calcd for $C_{50}H_{67}N_7O_6\cdot$2HCl: C, 64.23; H, 7.44; N, 10.49; Cl, 7.58. Found: C, 64.12; H, 7.61; N, 10.52; Cl, 7.38.

1,12,13,22-Tetraethyl-8,17-bis(hydroxypropyl)-2,7,18,23-tetramethylsapphyrin (2). 4,4′-Diethyl-5,5′-diformyl-3,3′-dimethyl-2,2′-bipyrrole[10] (544 mg, 2.0 mmol) and 2,5-bis(5-carboxy-3-(hydroxypropyl)-4-methylpyrrol-2-yl)methyl)-3,4-diethylpyrrole[20] (1.028 g, 2.0 mmol) were dissolved in absolute ethanol (2.0 L) with heating. The reaction mixture was allowed to cool to room temperature and *p*-toluenesulfonic acid monohydrate (1.5 g) was added. Air was vigorously bubbled through the reaction mixture for 4 days. The ethanol solvent was then removed using a rotorary evaporator and the product was isolated by column chromatography on silica gel using dichloromethane/methanol (1−5% v/v) as the eluent. The bishydrochloride salt was prepared by washing a dichloromethane solution of this sapphyrin with 1 N HCl to give a 78% (1.151 g) yield of the diprotonated product. ^{1}H NMR for **2**·2HCl (300 MHz, $CDCl_3$): δ −4.93 (2H, s, NH), −4.63 (1H, s, NH), −4.30 (2H, s, NH), 2.07 (6H, t, CH_2CH_3), 2.16 (6H, t, CH_2CH_3), 2.76 (4H, pentet, $CH_2CH_2CH_2$-OH), 4.03 (6H, s, CH_3), 4.06 (4H, t, $CH_2CH_2CH_2OH$), 4.20 (6H, s, CH_3), 4.59 (4H, q, CH_2CH_3), 4.68 (4H, q, CH_2CH_3), 4.77 (4H, t, CH_2-CH_2CH_2OH), 11.59 (2H, s, meso-H), 11.66 (2H, s, meso-H). ^{13}C NMR (80 MHz, $CDCl_3$): δ 13.15, 16.19, 17.84, 19.14, 21.85, 21.94, 25.04, 36.94, 62.25, 91.17, 97.53, 127.30, 128.38, 128.77, 131.77, 133.65, 134.46, 138.63, 140.54, 142.26, 143.88. UV−vis (free-base in MeOH): λ_{max} (ϵ) 443 (230 000), 573 (1 800), 618 (6 400), 669 (6 800). HRMS FAB calcd for $C_{42}H_{54}N_5O_2$ ($[M + H]^+$) 660.427751, obsd 660.428736. Anal. Calcd for $C_{42}H_{53}N_5O_2\cdot$2HCl·H_2O: C, 67.19; H, 7.65; N, 9.33; Cl, 9.44. Found: C, 67.25; H, 7.61; N, 9.41; Cl, 9.35.

8,18-Diethyl-1,13-bis[bis(hydroxyethyl)amino)carbonylmethyl]$^-$2,7,-12,17-tetramethylporphyrin (4). 3,13-Bis(carboxymethyl)-8,18-diethyl-2,7,12,17-tetramethylporphyrin[21] (269 mg, 0.5 mmol) was converted to its corresponding bis(*p*-nitrophenyl) ester.[21] This bis(*p*-nitrophenyl) ester (106 mg, 0.1356 mmol) was then added to dry pyridine (150 mL) under argon at 55 °C. This addition was followed by the addition of diethanolamine (104 mg, 1 mmol). The resulting reaction mixture was stirred for 4 days at 55 °C under argon in the dark. The pyridine was then removed *in vacuo*. A solution of aqueous NaOH (0.1 N, 25 mL) was added and the resulting suspension stirred for 15 min. The product obtained was then filtered off, washed with cold water (5 mL), and dried. The porphyrin **4** is freely soluble under acidic conditions (below pH 5). The free-base porphyrin was converted to the bishydrochloride salt by addition of 5% HCl (10 mL) followed by evaporation to dryness. The solid was then crystallized from water by adjusting the solution to pH 8. The mono- and dihydrochloride salts of the porphyrin **4** (**4**·2HCl and **4**·HCl) are freely soluble in water. The free-base product for elemental analysis was obtained *via* the addition of aqueous $NaHCO_3$, taking the resulting precipitate, washing it with water, and drying. ^{1}H NMR (300 MHz, $CDCl_3$, with 3% TFA v/v): δ −3.99 (4H, bs, N**H**), 1.72 (6H, t, CH_2CH_3), 3.48 (8H, t, NC**H**$_2$-CH_2OH), 3.62 (6H, s, CH_3), 3.65 (8H, t, NCH_2CH_2OH), 3.93 (6H, s, CH_3), 4.04 (4H, q, CH_2CH_3), 5.53 (4H, s, C**H**$_2$CON), 10.62 (2H, s, methine), 10.81 (2H, s, methine). ^{13}C NMR (80 MHz, $CDCl_3$, TFA): δ 11.37, 11.98, 16.48, 19.86, 31.58, 51.73, 59.37, 59.89, 98.10, 99.48, 112.26, 135.84, 138.11, 140.11, 141.04, 141.84, 142.23, 143.71, 158.65, 159.17, 171.62. FAB HRMS calcd for $C_{40}H_{54}N_6O_6$ $[M + H]^+$ 714.410484, obsd 714.411287. FAB HRMS calcd for $C_{40}H_{53}N_6O_6$ $[M]^+$ 713.402659, obsd 713.400834. UV−vis (**4**·2HCl in MeOH): λ_{max} (ϵ) 395 (120 000), 497 (9 300), 534 (7 100). Anal. Calcd for $C_{40}H_{54}N_6O_6\cdot 2H_2O$: C, 63.98; H, 7.79; N, 11.19. Found: C, 64.07; H, 7.84; N, 11.22.

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Results and Discussion

Equilibria of Interest. As was true for our earlier study of halide anion binding to diprotonated sapphyrin,[6a,13] the objectives of the present study are basically 3-fold: first, obtain structural information allowing for an assessment of how phosphate anions might be bound to diprotonated sapphyrin in the solid state; second, determine appropriate affinity constants so as to establish the extent to which diprotonated sapphyrin functions as a phosphate anion receptor under solution-phase conditions; and, third, analyze, to the extent possible, the nature of the interaction(s) between phosphate anions and diprotonated sapphyrin in solution and determine if these correspond to what is seen in the solid state. However, because much of our underlying interest in phosphate anion chelation derives from a desire to prepare sapphyrin-based carriers for the through-membrane transport of mono- and dianionic nucleotides at neutral pH, we also became interested in assessing the extent to which the monoprotonated form of sapphyrin (the form that is the dominant species at neutral pH[13]) might be capable of acting as a phosphate anion receptor. Thus, the following equilibria are of interest in terms of the present study:

$$[2H\cdot Sap]^{2+} \rightleftarrows [H\cdot Sap]^{+} + H^{+} \qquad K_{a1} \tag{1}$$

$$[H\cdot Sap]^{+} \rightleftarrows Sap + H^{+} \qquad K_{a2} \tag{2}$$

$$[2H\cdot Sap]^{2+} + X^{n-} \rightleftarrows [[2H\cdot Sap]^{2+}\cdot X^{n-}]^{(2-n)+} \qquad K_{21} \tag{3}$$

$$([2H\cdot Sap]\cdot X)^{(2-n)+} + X^{n-} \rightleftarrows [[2H\cdot Sap]^{2+}\cdot 2X^{n-}]^{(2-2n)+} \qquad K_{22} \tag{4}$$

$$[H\cdot Sap]^{+} + X^{n-} \rightleftarrows [[H\cdot Sap]^{+}\cdot X^{n-}]^{(1-n)+} \qquad K_{11} \tag{5}$$

wherein Sap, $[H\cdot Sap]^+$, and $[2H\cdot Sap]^{2+}$ refer to the free-base, monoprotonated, and diprotonated forms of sapphyrin, respectively, and X^{n-} represents some anionic species such as a halide anion ($n = 1$) or an anionic phosphate entity such as phenyl phosphate for which $n = 1$ or 2, depending on the pH. The terms K_{a1} and K_{a2} refer to the acid dissociation constants for the loss of one and two protons, respectively, from the diprotonated form of sapphyrin; and, the terms K_{21}, K_{22}, and K_{11} refer to the association constants for the binding of the first and second molar equivalents of anionic substrate, X^{n-}, to the diprotonated form of sapphyrin and the binding of a single anionic substrate to the monoprotonated form of sapphyrin, respectively.

In addition to the above equilibria, it is also of interest to consider various anion exchange processes. Among those most relevant to the present study are the following:

$$[[2H\cdot Sap]^{2+}\cdot X^{n-}]^{(2-n)+} + Y^{m-} \rightleftarrows [[2H\cdot Sap]^{2+}\cdot Y^{m-}]^{(2-m)+} + X^{n-} \qquad K_{2ex} \tag{6}$$

$$[[H\cdot Sap]^{+}\cdot X^{n-}]^{(1-n)+} + Y^{m-} \rightleftarrows [[H\cdot Sap]^{+}\cdot Y^{m-}]^{(1-m)+} + X^{n-} \qquad K_{1ex} \tag{7}$$

where X^{n-} and Y^{m-} are different anions being subject to possible exchange, and the terms K_{2ex} and K_{1ex} are meant to denote the simple one-to-one anion exchange processes in the case of the

monomeric anion complexes of the di- and monoprotonated forms of sapphyrin, respectively.

In a previous solution-phase study,[13] it was found that only a single anionic halide substrate (i.e., F^-, Cl^-, or Br^-) was bound with appreciable affinity to the diprotonated form of sapphyrin **3**, $[2H\cdot\mathbf{3}]^{2+}$, in either dichloromethane or methanol. Thus, the equilibrium process represented by eq 4 could be effectively ignored, in spite of the fact that chloride anion was found to form a 2:1 "sitting atop" complex with $[2H\cdot\mathbf{3}]^{2+}$ in the solid state. In the present instance, therefore, it was also considered likely that only a single anionic phosphate entity would bind to the diprotonated forms of sapphyrins **1**−**3**. However, depending on pH and/or the degree of proton transfer from/to the protonated/neutral forms of sapphyrin, phenyl phosphate and phosphate can be considered as bearing negative charges as high as 2 or 3, respectively. This greater charge density (represented by a value of $n > 1$ in the above equations) and the increased electrostatic attraction it might engender could then favor the equilibrium of eq 4 under conditions where it would not be important for simple halide anion complexation. As a result, several qualitative tests were carried out to determine whether or not the diprotonated forms of sapphyrins **1**−**3** might, or might not, be binding phosphorylated substrates with 1:2 stoichiometry in solution. These include both extraction experiments and FAB MS analyses. Before turning to a discussion of these experiments, however, a consideration of the solid-state structures is in order. This solid-state structural information provides the basic proof that diprotonated sapphyrins can bind phosphate species, at least under certain well-defined conditions.

Description of Solid-State Structures. X-ray structures of four phosphate complexes have been obtained, namely the 1:1 inner-sphere, cationic complex of formally monobasic phosphoric acid (dihydrogen phosphate) with diprotonated sapphyrin **2**, $[[2H\cdot\mathbf{2}]^{2+}\cdot[H_2PO_4]^-]^+$, the analogous neutral 1:1 complex formed between dibasic phosphoric acid (hydrogen phosphate) and diprotonated sapphyrin **2**, $[2H\cdot\mathbf{2}]^{2+}\cdot[HPO_4]^{2-}$, the 2:1 inner-sphere complex of monobasic phenyl phosphate with the diprotonated form of sapphyrin **3**, $[2H\cdot\mathbf{3}]^{2+}\cdot 2[C_6H_5OP(O)(OH)O]^-$, and the 2:1 complex of diphenyl phosphate with diprotonated sapphyrin **2**, $[2H\cdot\mathbf{2}]^{2+}\cdot 2[(C_6H_5O)_2P(O)O]^-$. Representations of these structures are given in Figures 1−6, with selected bond distances and angles being given in the captions to these figures.

The latter two complexes bear considerable structural resemblance to the earlier reported structure of the dichloride complex of $[2H\cdot\mathbf{3}]^{2+}$.[12] For instance, just as is true for the two chloride anions in the structure of $[2H\cdot\mathbf{3}]^{2+}\cdot 2Cl^-$, in both phosphate ester structures, the key ligated oxygen atoms of the phosphate ester monoanions are held above and below the sapphyrin plane in what is formally a "sitting atop" sense. As important, and again in analogy to the structure of $[2H\cdot\mathbf{3}]^{2+}\cdot 2Cl^-$, these "upper" and "lower" oxygen atoms are held in place by three and two hydrogen bonds, respectively, and are displaced off the normal through the center of the mean N_5 sapphyrin plane. Unlike the chloride anion complex, however, there is an overall shape associated with the anionic portion of the receptor−substrate ensemble. For example, in the phenyl phosphate structure (Figure 4), the phenyl portions of the molecular anion are found to lie over a portion of the aromatic sapphyrin skeleton and assume near-planar conformations with one of the phenyl rings making contact with the sapphyrin macrocycle at distances that range from 3.5 to 3.6 Å with a dihedral angle of 2° while the other is characterized by a dihedral angle of 11° and contacts ranging between 3.4 and 3.9 Å. In both cases, it is clear that at least some of the contact distances are close to those expected for *bona fide* van der Waals contact (ca. 3.4 Å[22]). On the other hand, interestingly, the phenyl subunits in the analogous 2:1 diphenyl phosphate−sapphyrin **2** complex do not reside anywhere in the macrocycle plane (Figure 6). This leads us to suggest that although there is some correspondence between these two structures, especially where the sapphyrin-to-anion binding features are concerned, there remain some subtle differences that can only be rationalized in terms of the overall anion shape and/or mode of crystallization. In this context, it is perhaps interesting to note that the structure of $[2H\cdot\mathbf{2}]^{2+}\cdot 2[(C_6H_5O)_2P(O)O]^-$ reveals a pure 1:2 sapphyrin-to-phosphate complex (Figure 6), whereas that of the corresponding phenyl phosphate complex, $[2H\cdot\mathbf{3}]^{2+}\cdot 2[C_6H_5OP(O)(OH)O]^-$, displays an infinite hydrogen bonding array established by, among other things, phenyl phosphate-to-phenyl phosphate interactions in-

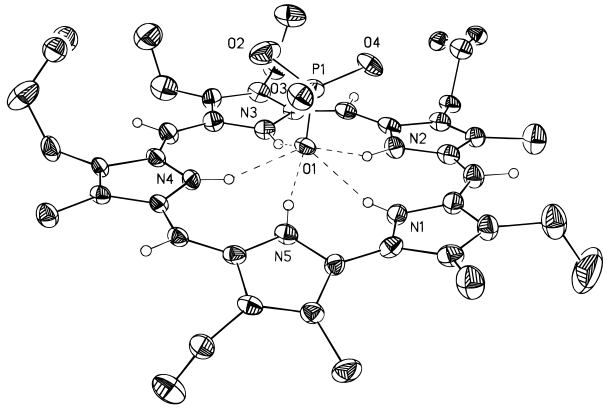

Figure 1. View of sapphyrin salt $\mathbf{2}\cdot[H_3PO_4]_3\cdot 0.68H_2O$, corresponding to the 1:1 cationic inner-sphere complex $[[2H\cdot\mathbf{2}]^{2+}\cdot[H_2PO_4]^-]^+$, showing the partial atom labeling scheme. Thermal ellipsoids are scaled to the 30% probability level. Dashed lines are indicative of a H-bonding interaction. The relevant H-bond distances (Å) and angles (deg) are the following: N1−H1N···O1, N···O 2.849(9), H···O 2.046(9), N−H···O 147.8(8); N2−H2N···O1, N···O 2.928(9), H···O 2.073(9), N−H···O 158.3(8); N3-H3N···O1, N···O 2.816(9), H···O 1.968(9), N−H···O 156.3(8); N4−H4N···O1, N···O 2.836(8), H···O 1.988(8), N−H···O 156.4(7); N5−H5N···O1, N···O 2.865(8), H···O 1.972(8), N−H···O 171.2(8). O1 is approximately centered above the N_5 plane and is offset by 0.16 Å from a line perpendicular to the N_5 plane passing through the center of the five nitrogen atoms.

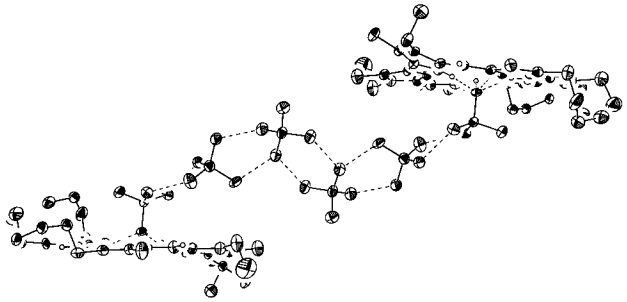

Figure 2. View of sapphyrin salt $\mathbf{2}\cdot[H_3PO_4]_3\cdot 0.68H_2O$, corresponding to the 1:1 cationic inner-sphere complex $[[2H\cdot\mathbf{2}]^{2+}\cdot[H_2PO_4]^-]^+$, showing the phosphate aggregation in the crystal lattice. Six phosphate groups are linked in an array by strong hydrogen bonds. This array is terminated by a sapphyrin macrocycle at each end. The aggregate shown lies around an inversion center at $^1/_2$, $^1/_2$, $^1/_2$. The hydrogen atoms on the phosphate oxygens were not observed. The hydrogen bonding was inferred from the close O···O contacts which ranged from 2.47 to 2.56 Å. These aggregates are H-bound to adjacent aggregates via H bonds involving the side chains of the macrocycle and the phosphate oxygen atoms.

(22) Hunter, C. A.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1990**, *112*, 5525−5534.

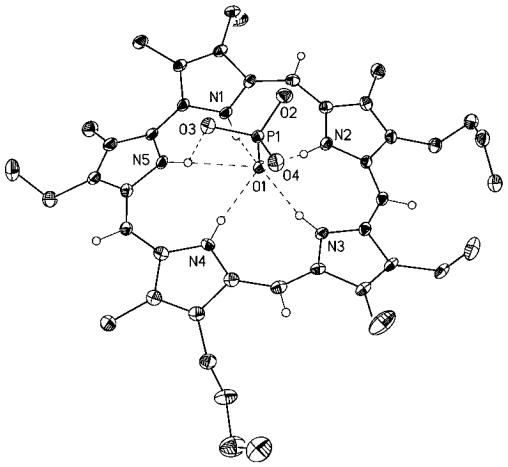

Figure 3. View of sapphyrin salt **2**·$[H_3PO_4]$·H_2O, corresponding to the 1:1 neutral complex $[2H·\mathbf{2}]^{2+}$·$[HPO_4]^{2-}$, showing the partial atom labeling scheme. Thermal ellipsoids are scaled to the 30% probability level. The coordination is 1:1 as in $[[2H·\mathbf{2}]^{2+}·[H_2PO_4]^{-}]^{+}$ but with a different mode of binding. In the present instance, there is a bifurcated H bond involving O1 which resides in the macrocyclic cavity and O3 with H5. Dashed lines are indicative of the H-bonding interaction. The relevant H-bond distances (Å) and angles (deg) for O1 are the following: N1−H1···O1, N···O 2.824(6), H···O 1.99(5), N−H···O 160(4); N2−H2···O1, N···O 2.797(6), H···O 1.78(6), N−H···O 177(5); N3−H3···O1, N···O 2.820(6), H···O 1.79(6), N−H···O 174(5); N4−H4···O1, N···O 2.889(6), H···O 1.96(5), N−H···O 173(4); N5−H5···O1, N···O 3.032(6), H···O 2.37(5), N−H···O 129(4). Those for O3 are the following: N5−H5···O3, N···O 2.720(6), H···O 1.83(6), N−H···O 163(5). The bifurcated H-bond angle about O3···H5···O1 is 67(2)°. O1 is approximately centered above the N_5 plane and is offset by 0.26 Å from a line perpendicular to the N_5 plane passing through the center of the five nitrogen atoms.

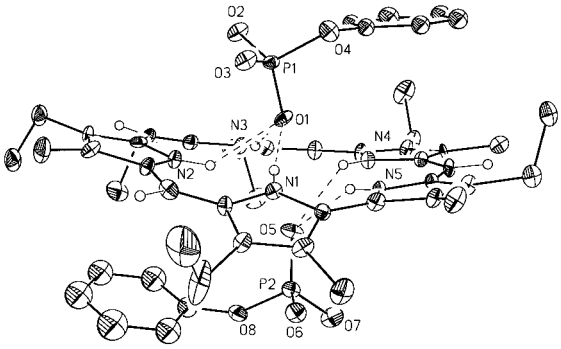

Figure 4. View of sapphyrin salt **3**·3$[C_6H_5OPO(OH)_2]$, corresponding to the neutral 1:2 complex $[2H·\mathbf{3}]^{2+}$·2$[C_6H_5OP(O)(OH)O]^{-}$, showing the partial atom labeling scheme. Thermal ellipsoids are scaled to the 20% probability level. Most hydrogen atoms have been excluded for clarity. The H-bonding interactions are indicated by dashed lines. Relevant H-bond distances (Å) and angles (deg) are the following: N1−H1N···O1, N···O 3.02(1), H···O 2.13(1), N−H···O 173(1); N2−H2N···O1, N···O 2.90(2), H···O 2.18(2), N−H···O 137(1); N3−H3N···O1, N···O 2.84(1), H···O 1.98(1), N−H···O 158(1); N4−H4N···O5, N···O 3.06(1), H···O 2.31(1), N−H···O 140.6(9); N5−H5N···O5, N···O 2.84(1), H···O 2.05(1), N−H···O 147(1).

volving the "extra" two molecular equivalents of phenyl phosphoric acid present in the unit cell (Figure 5). Such aggregation effects have been previously observed both in organic solvents (e.g., chloroform,[4z,ab] acetonitrile[4ae]) and in the solid state[4aj,13b] and are discussed explicitly further on in this report.

The structure of $[[2H·\mathbf{2}]^{2+}·[H_2PO_4]^{-}]^{+}$ (Figure 1), in contrast to the above, has no direct precedent in the previous protonated sapphyrin−halide anion structures and, apparently, is also without parallel in the phosphate anion chelation literature. What

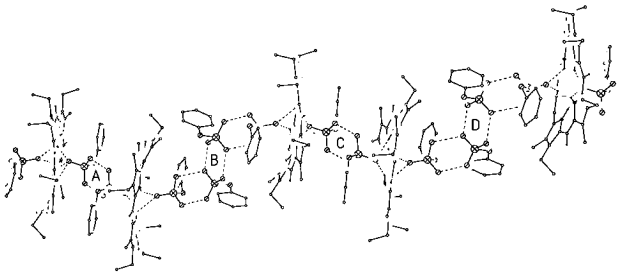

Figure 5. View of sapphyrin salt **3**·3$[C_6H_5OPO(OH)_2]$, corresponding to the neutral 1:2 complex $[2H·\mathbf{3}]^{2+}$·2$[C_6H_5OP(O)(OH)O]^{-}$. The phenyl phosphate groups form H-bound aggregates in the unit cell which form an infinite array parallel to 0,−1,1. Two phenyl phosphate groups are directly bound to opposing sides of the macrocycle. Adjacent complexes, related by an inversion center at $0,{}^{3}/_{2},0$, form H-bound dimers (A). The phenyl phosphoric acid molecule forms a hydrogen bound dimer to its inversion relative across $0,1,{}^{1}/_{2}$ (B). This dimer forms a bridge linking the macrocyclic dimers centered at A and C $(0,{}^{1}/_{2},1)$. D is located at $0,0,{}^{3}/_{2}$. The hydrogen atoms of the phenyl phosphate groups were not located. Hydrogen-bonding interactions are inferred from very close O···O contacts which range between 2.43(1) and 2.59(1) Å.

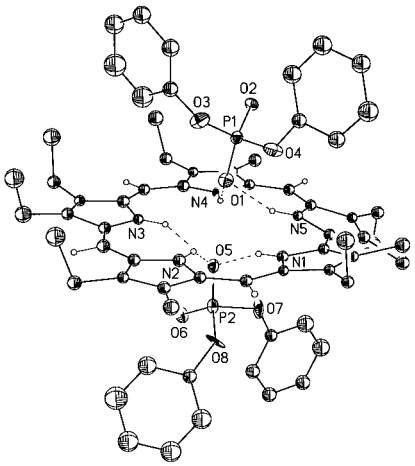

Figure 6. View of sapphyrin salt **2**·2$[(C_6H_5O)_2PO(OH)]$, corresponding to the neutral 1:2 complex $[2H·\mathbf{2}]^{2+}$·2$[(C_6H_5O)_2P(O)O]^{-}$, showing the partial atom labeling scheme. The P and O atoms were refined anisotropically, all other non-H atoms were refined isotropically and are shown scaled to the 20% probability level. One diphenyl phosphonate group is H-bound to three NH groups of the macrocycle, while the second is H-bound to two NH groups. The relevant H-bond distances (Å) and angles (deg) are the following: N1−H1a···O5, N···O 2.82(2), H···O 1.95(2), N−H···O 162(1); N2−H2a···O5, N···O 2.76(2), H···O 1.96(2), N−H···O 147(2); N3−H3a···O5, N···O 2.86(2), H···O 2.04(2), N−H···O 150(1); N4−H4a···O1, N···O 2.82(2), H···O 2.04(2), N−H···O 144(1); N5−H5a···O5, N···O 2.82(2), H···O 1.96(2), N−H···O 158(2).

makes this structure so unique is that it is characterized by a single bound dihydrogen phosphate counter anion tethered *via* five hydrogen bonds to a diprotonated sapphyrin receptor. This results in an umbrella-like arrangement in which the other three oxygen atoms of the bound $H_2PO_4^{-}$ counter anion are splayed out like spokes on a wheel.

In this structure, the bound oxygen is 1.22 Å above the mean N_5 sapphyrin plane and offset from its perpendicular by ca. 0.16 Å. The central phosphorus is 2.62 Å from this same N_5 plane. Nitrogen-to-bound oxygen (O1) distances (Å) are 2.849(9) (N1), 2.928(9) (N2), 2.816(9) (N3), 2.836(8) (N4), and 2.865(8) (N5), values that are completely consistent with the proposed hydrogen bonding interactions. The actual protons, however, were not localized in this structure (or in the ones discussed immediately above). Thus, the assignment of this structure as consisting of a cationic complex between diprotonated sapphyrin and mono-

basic phosphoric acid is true only in a formal sense; it could be formulated as well, for instance, as being a neutral complex formed between monoprotonated sapphyrin and free phosphoric acid. Arguing against this latter interpretation, however, is the finding that the corresponding 1:1 phosphate−sapphyrin complex, $[2H\cdot\mathbf{2}]^{2+}\cdot[HPO_4]^{2-}$ (Figure 3), formed following extractions carried out at pH 6.0, is best formulated as being the mixed salt of diprotonated sapphyrin and dibasic phosphoric acid. In this instance, the actual pyrrolic NH protons were localized in the difference map. Further, in this case, a second protonated sapphyrin-to-phosphorus oxygen (O3···N5 2.715 Å) interaction is seen that is not present in the structure of $[[2H\cdot\mathbf{2}]^{2+}\cdot[H_2PO_4]^-]^+$ (O3···N5 3.148 Å); this is as one might intuit for a complex formed between a dicationic receptor and a dianionic phosphate species.

The availability of structural information for two sapphyrin phosphate complexes, obtained as the result of crystallizations carried out subsequent to extractions performed at two different pH values (cf. Experimental Section), allows other interesting differences to be revealed. Prime among these are differences in *overall* phosphate-to-sapphyrin stoichiometry. While both structures, corresponding to complexes $[[2H\cdot\mathbf{2}]^{2+}\cdot[H_2PO_4]^-]^+$ (Figure 1) and $[2H\cdot\mathbf{2}]^{2+}\cdot[HPO_4]^{2-}$ (Figure 3), are of a 1:1 nature as far as the *direct* interactions with the sapphyrin nucleus are concerned, in the case of the former cationic complex ($[[2H\cdot\mathbf{2}]^{2+}\cdot[H_2PO_4]^-]^+$), two more per sapphyrin equivalents of H_3PO_4 are found hydrogen bound in the unit cell (Figure 2). On the basis of charge considerations, one of these is considered to be free phosphoric acid whereas the other is assigned as being $H_2PO_4^-$. This second needed counter anion, while again displaying evidence for phosphate-to-phosphate aggregation in the solid state (see above), is not proximate to the sapphyrin core and thus plays no role in defining the details of complexation. As a result, one is left, as was true in the previously-reported fluoride anion complex, $[[2H\cdot\mathbf{3}]^{2+}\cdot F^-]^+$,[6a,13] with a system in which only a single negative anion is bound to an overall dication in the solid state and an indication, no matter how subjective, that the equilibrium of eq 3 might serve to define the related chemistry in the solution phase. Interestingly, no such "extra" phosphoric acid equivalents were found in the case of the second complex, $[2H\cdot\mathbf{2}]^{2+}\cdot[HPO_4]^{2-}$.

Issues of Aggregation. There has been considerable discussion about multiple equilibria as a complicating feature associated with phosphate anion recognition.[4z,ab,ae] It has been clearly shown that different phosphodiesters have a tendency to form dimeric structures in chloroform[4z,ab] and acetonitrile.[4ae] In our case, we have obtained direct evidence for phosphate-to-phosphate interactions in single crystals of complexes $[2H\cdot\mathbf{3}]^{2+}\cdot 2[C_6H_5OP(O)(OH)O]^-$ and $[[2H\cdot\mathbf{2}]^{2+}\cdot[H_2PO_4]^-]^+$ (see discussion immediately above). We have also seen evidence of multiple equilibria, specifically phosphodiester dimerization, during the course of NMR titrations involving sapphyrin **2** and diphenyl phosphate in methanol-d_4 (cf. supporting information). Fortunately, no evidence for such complicating equilibria was observed in the case of titrations involving phenyl phosphate or dihydrogen phosphate in methanol-d_4; here, as described in the ensuing subsection, clean binding isotherms were obtained that, depending on the conditions, were readily interpretable in terms of the formation of either 1:1 or 2:1 phosphate-to-protonated sapphyrin complexes. Apparently, the higher polarity of methanol, relative to chloroform or acetonitrile, and the lower hydrophobicity of these two phosphate anions, as compared to diphenyl phosphate, is sufficient to prevent significant phosphate-to-phosphate aggregation under the conditions of our experiments (methanol-d_4, 0.5−3 mM, ambient temperature). In the event, the absence of such complicating interactions facilitated the design and interpretation of quantitative analyses (*vide infra*).

Also facilitating the design and interpretation of quantitative binding analyses is a choice of conditions (such as solvent, concentration, and choice of receptor) that precludes appreciable receptor−receptor aggregation. Such self-association processes are known to plague many salt-type receptor systems[4ab] and are a known phenomenon in sapphyrin chemistry.[17,23,24] It was found, however, that sapphyrin **2** is not only readily soluble in methanol, but remains largely monomeric in this solvent over a wide range of concentrations. For the free-base and monohydrochloride forms, sapphyrin−sapphyrin aggregation (dimerization) becomes appreciable at ca. 1 mM in the absence of phosphate as indicated by the presence of a Soret-like absorption band at $\lambda_{max} \approx 420$ nm (ascribed to the dimeric form of sapphyrin) as well as at $\lambda_{max} \approx 445$ nm (assigned to the monomer).[25] For the bishydrochloride salt (and the bis-(dihydrogen phosphate salt)), no evidence of aggregation was obtained even at concentrations as high as 3 mM (as determined by good adherence to Beer's law behavior). Thus, under the conditions of the NMR and UV−vis titrations described below, the interactions of sapphyrin **2** with various putative phosphate substrates could generally be studied without concern for the effects of potentially complicating self-aggregation equilibria. This was especially true for those studies that involved use of the bishydrochloride salt, **2**·2HCl, and for those where the concentrations of the other sapphyrin species, namely **2**·HCl and **2**, were kept low (i.e., UV−vis, early part of the ^{31}P and ^1H NMR titration profiles).

Unfortunately, such simplicity was not manifest in the case of the water-soluble system **1** when studied at neutral pH. Here, in a range of buffer systems, it was found that the sapphyrin existed in a highly aggregated form (as evidenced by characteristic absorption bands with λ_{max} of 400 nm[25]). However, as detailed more fully in the ensuing paper, it was found that addition of phosphate-containing anions to these aggregates leads to the formation of first a phosphate-bound dimeric sapphyrin species ($\lambda_{max} \approx 420$ nm[25]) followed by the generation of monomerized sapphyrin−phosphate complexes ($\lambda_{max} \approx 450$ nm[25]) at very high phosphate-to-sapphyrin ratios. While such changes could be used as the basis for quantitative analyses, in general the presence these multiple equilibria complicated efforts to determine phosphate-to-sapphyrin association constants in water.

Solution-Phase Studies. Evidence for phosphate anion complexation under noncrystalline conditions came from both fast atom bombardment mass spectrometric (FAB MS) and solvent−solvent extraction studies. In the first of these, the free-base sapphyrins **2** or **3** were mixed with phosphoric acid, phenyl phosphate (monosodium salt), phenylphosphonic acid, diphenyl phosphoric acid, or the acid form of AMP in methanol or methanol−water (1:1) in either a 1:1 or 2:1 stoichiometric ratio (phosphate-to-sapphyrin). Then, after taking the resulting solutions to dryness, the resulting product or product mixtures were analyzed by positive ion FAB MS using nitrobenzyl alcohol as the matrix. The results, which are summarized in

(23) Maiya, B. G.; Cyr, M.; Harriman, A.; Sessler, J. L. *J. Phys. Chem.* **1990**, *94*, 3597−3601.

(24) Král, V.; Andrievski, A.; Sessler, J. L. *J. Am. Chem. Soc.* **1995**, *117*, 2953−2954.

(25) As discussed previously[23] and in the ensuing paper,[17b] visible spectroscopy provides a very convenient means of assessing sapphyrin−sapphyrin aggregation: The characteristic Soret-type absorbance band of sapphyrin (free-base, mono- or diprotonated) appears at 445−450 nm when it is monomeric, at ca. 420 nm when it is dimeric, and at ca. 400 nm when it is more highly aggregated.

Table 2. Composition of 1:1 Sapphyrin (**2** and **3**)−Phosphate Complexes As Determined by HR FAB MS

			HR FAB MS	
sapphyrin	phosphate	composition[a]	calcd	found
3	phenylphosphonic acid	$[(C_{40}H_{49}N_5)H_2]\cdot C_6H_6PO_3$	758.419905	758.420073
3	diphenylphosphate	$[(C_{40}H_{49}N_5)H_2]\cdot C_{12}H_{10}PO_4$	850.446119	850.445856
2	phosphoric acid	$[(C_{42}H_{53}N_5O_2)H_2]\cdot H_2PO_4$	758.404649	758.405254
2	phenylphosphonic acid	$[(C_{42}H_{53}N_5O_2)H_2]\cdot C_6H_6PO_3$	818.441034	818.439615
2	phenyl phosphate monosodium salt	$[(C_{42}H_{53}N_5O_2)H_2]\cdot NaC_6H_5PO_4$	856.417893	856.417927
2	AMP	$[(C_{42}H_{53}N_5O_2)H_2]\cdot C_{10}H_{13}N_5PO_7$	1007.490838	1007.492544
2	AMP, CMP, GMP (1:1:1 ratio)[c]	**2**·AMP (95%)[b]	1006.483013	1006.481323[d]
		2·CMP (81%)[b]	983.479605	983.482851[d]
		2·GMP (90%)[b]	1023.485753	1023.485062[d]

[a] Composition corresponds to $[[2H\cdot Sap]^{2+}\cdot[phosphate]^-]^+$. [b] Refers to observed ion intensities relative to base peak. [c] Following extraction of sapphyrin **2** with an equimolar mixture of nucleotides at pH 6.0. [d] Peaks corresponding to $[M + H]^+$ species were also observed (1007.495; 984.487; 1024.4889).

Table 2, were in all cases consistent with an ionized 1:1 sapphyrin-to-phosphate complex of the general form $[[2H\cdot Sap]^{2+}\cdot[ROP(O)(OH)O]^-]^+$, where Sap denotes the free-base form of either sapphyrin **2** or **3**.[26] In fact, in no cases were peaks corresponding to 2:1 phosphate-to-sapphyrin adducts observed. Thus, to the extent that such an extrapolation is reasonable, these mass spectrometric studies argue against 2:1 inner-sphere complexes, such as those shown in Figures 4 and 6, being the dominant species in solution. Nonetheless, it is to be appreciated that strictly speaking these mass spectrometric analyses give no direct evidence for the composition of solution-phase phosphate anion binding *per se*. Thus, the extraction experiments were undertaken.

Extraction experiments were carried out by monitoring the extent to which phenylphosphonic acid (or its corresponding basic forms) was found to partition between dichloromethane and water at pH 5.6 and 1.58 in the presence of sapphyrins **2** and **3** or porphyrin **5**. At these two pH values, the sapphyrin in question was expected to be either mono- and diprotonated and thus capable of binding 1 or 2 equiv of phosphate, respectively. This was indeed found to be the case: At pH 5.6, the two sapphyrins in question served to "pull into" the organic layer 1.0 ± 0.06 equiv of phenylphosphonate (as judged by NMR integrations; cf. Experimental Section) whereas 2.0 ± 0.04 equiv were extracted into dichloromethane when the same experiments were carried out at pH 1.58. The same results were obtained for phenyl phosphate. In no cases, however, was there any evidence of any significant extraction when porphyrin **5** was used at either of these two pH extremes. Nor was there evidence of any appreciable partitioning of phenylphosphonate out of water into dichloromethane if no macrocycle, either porphyrin or sapphyrin, was used. Thus, taken together, these extraction experiments served to confirm qualitatively the notion obtained earlier from various model through-membrane transport studies[15,16] that appropriate, protonated sapphyrins are indeed capable of binding phosphate-derived anions in organic solution.

NMR Analyses. More direct evidence for phosphate anion binding in solution came from NMR analyses. Here, both ^1H and ^{31}P NMR methods were used. In both instances, the key predicative idea was that the aromatic ring current of the sapphyrin nucleus, shifting to higher field the signals of substrates held above it, would provide a "handle" that would allow us to monitor the formation of a complex. Specifically, large upfield shifts were expected in both the ^1H and ^{31}P spectra of sapphyrin-bound substrates, such as phenyl phosphate and phenylphosphonate, that incorporate both these nuclei. As discussed in greater detail below, this indeed proved to be the case.

^{31}P NMR Spectra. Initial NMR studies were performed in D_2O and DMSO-d_6 as the solvents. Under these conditions, signals for both complexed and uncomplexed forms were observed when sapphyrin **1** was mixed with, e.g., 0.33 molar equiv of phosphoric acid. For the signals ascribed to the sapphyrin−phosphate complex, upfield shifts of 7.1 and 7.5 ppm were recorded in D_2O and DMSO-d_6, respectively, at these molar ratios. In the case of the corresponding studies carried out using 0.33 molar equiv of phenylphosphonic acid (relative to **1**), upfield shifts of 10.5 ppm were observed in D_2O. Such large upfield shifts, which are thought to reflect the large ring current associated with the aromatic sapphyrin, are, of course, readily interpretable in terms of complex formation. They thus provide qualitative support for the notion that sapphyrins bind phosphates well in aqueous or other highly polar media (*viz.*, DMSO-d_6). Unfortunately, we were unable to obtain more quantitative conclusions (i.e., binding affinities) from these experiments: Efforts to carry out full titrations in these solvents were precluded by the low solubility of the free-base form of **1** and its known proclivity for self-association (i.e., dimerization, aggregation).

In contrast to the above, it was found that complete binding titrations could be carried out in methanol-d_4 using several phosphate and phosphonate species as the substrates and sapphyrin **2** as the receptor. Here, as described in the Experimental Section, increasing concentrations of sapphyrin **2** were added to initial phosphate or phosphonate solutions (as the free-acid and monobasic potassium, tetrabutylammonium, or triethylammonium salts) of 0.5 to 3 mM concentration and the changes in the ^{31}P chemical shifts recorded. Interestingly, in this solvent system, only averaged signals for the ^{31}P resonances were observed. Nonetheless, very large upfield shifts for these signals were observed at or near saturation (Figures 7 and 8). This was true whether phenylphosphonate, phenyl phosphate, or dihydrogen phosphate was used as the anion.[27,28] In fact, in all cases, the extent of the induced upfield shift was very large compared to anything previously seen in the literature.

(26) Although the results of high-resolution FAB MS analysis are consistent with 1:1 cationic complexes of the generalized formula $[[2H\cdot Sap]^{2+}\cdot[ROP(O)(OH)O]^-]^+$, the known tendency of big, porphyrin-like systems to lose and gain protons (and H atoms) under these ionization conditions (see, for example: Sessler, J. L.; Johnson, M. R.; Creager, S. E.; Fettinger, J. C.; Ibers, J. A. *J. Am. Chem. Soc.* **1990**, *112*, 9310−9329) makes a distinction between 1:1 complexes of the $[[2H\cdot Sap]^{2+}\cdot[ROP(O)(OH)O]^-]^+$ and $[H\cdot Sap]^+\cdot[ROP(O)(OH)O]^-$ types difficult (corresponding to the equilibria of eqs 3 and 5, respectively) by this means.

(27) Detailed studies were not made using diphenyl phosphate as this particular substrate was found to undergo significant self-association at the concentrations studied ($\geq$0.5 mM) even in methanol-d_4.

(28) The magnitude of the shifts was always greater in the case of phenylphosphonate than in the case of the dihydrogen phosphate which we interpret in terms of better binding interactions with phenylphosphonate. These we suggest could result from an extra stabilization arising from favorable $\pi-\pi$ stacking effects present in the case of the phenylphosphonate complex and would be analogous to those seen in the solid-state structure of the 2:1 phenyl phosphate−sapphyrin complex shown in Figure 4.

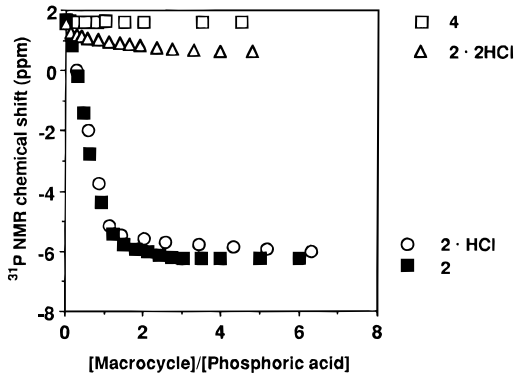

Figure 7. Changes in phosphate ^{31}P NMR signal observed as phosphoric acid is titrated with increasing quantitities of sapphyrin **2**, sapphyrin monohydrochloride (**2**·HCl), sapphyrin bishydrochloride (**2**·2HCl), and porphyrin **4** in methanol-d_4. For experimental conditions, see text.

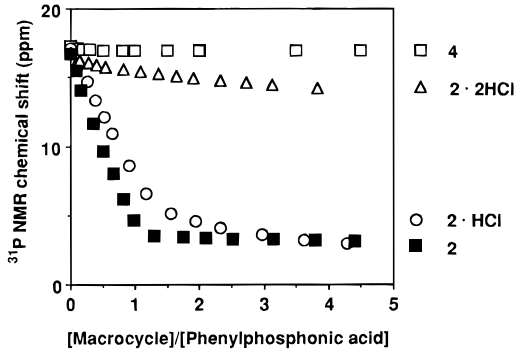

Figure 8. Changes in phosphate ^{31}P NMR signal observed as phenylphosphonic acid is titrated with increasing quantitities of sapphyrin **2**, sapphyrin monohydrochloride (**2**·HCl), sapphyrin bishydrochloride (**2**·2HCl), and porphyrin **4** in methanol-d_4. For experimental conditions, see text.

For instance, whereas the induced ^{31}P NMR chemical shifts engendered by porphyrins are usually small (i.e., on the order of −4 ppm in chloroform-d[29a] but less than −1 ppm in water[29b]), shifts of nearly −7 ppm for phosphoric acid and more than −13 ppm for phenylphosphonic acid are observed with free-base sapphyrin **2** in methanol-d_4 at the point in the titrations where 1 molar equiv of sapphyrin had been added.[30] Likewise, at the point in the titration where 2.0 molar equiv of sapphyrin **2** had been added to a solution of tetrabutylammonium dihydrogen phosphate in CD_2Cl_2, a 16.0 ppm upfield shift in the phosphate ^{31}P NMR resonance was observed; under identical conditions and at identical molar ratios, the control porphyrin engendered only a 0.20 ppm upfield shift. Thus, although not a formal proof,[4aa,30] these spectroscopic findings are clearly consistent with the fundamental premise of this paper, namely that the protonated sapphyrins will bind phosphorylated substrates under conditions where the porphyrins will not.

When the above titrations were run using free-base sapphyrin **2** and the free-acid forms of the phosphate substrates in question, they gave K_{11} and K_{22} directly. They could also be run in such a way as to give K_{21} values *via* anion exchange. For these latter analyses, the titrations were run using **2**·2HCl, for which a K_{21} value of 100^{-1} M is assumed (based on prior work with 3·2HCl).[13]

Table 3. Affinity Constants for the Binding of Phosphate and Phenylphosphonate to Sapphyrin **2** in Methanol-d_4 at Ambient Temperature As Determined from ^{31}P NMR, ^{1}H NMR, and Visible Spectroscopic Measurements

substrate	method[a]	K_{11}[b] (M^{-1})	K_{21}[b] (M^{-1})	K_{22}[b] (M^{-1})
phosphoric acid[d]	^{31}P NMR[a]	12600	13900	500
phenyphosphonic acid	^{31}P NMR[a]	16900	18200	3150
	^{1}H NMR	16800	17200	3080
	vis[c]			3650

[a] Binding constants K_{11} and K_{22} were determined from curve fitting analyses carried out using standard curve fitting programs (cf. ref 18). K_{21} values were determined from exchange experiments that involved replacing the initially-present chloride counter anion by the phosphate or phosphonate anion in question; a K_{21} value for Cl^- of 100 M^{-1} (ref 13) was used. [b] K_{11}, K_{21}, and K_{22} values are averages of three independent measurements; the estimated error is less than ±5%. [c] Determined from UV−vis spectroscopic titrations carried out in MeOH. A standard curve-fitting program (cf. ref 18) was used for data analysis. [d] Under the conditions of the experiment (0.2−3 mM) no dimerization of sapphyrin **2**−phosphate was observed. Likewise, no evidence for phosphate dimerization was observed under these conditions.

In understanding these titration experiments, it is important to appreciate that, from a phenomenological perspective, two basic types of phosphate binding are being observed. The first involves anion binding concomitant with proton transfer and occurs, for instance, when the free-base form of sapphyrin is added to various phosphate free-acids. The second involves anion exchange and is relevant when, for instance, the bound chloride counter anion originally present in $[[2H\cdot\mathbf{2}]^{2+}\cdot Cl^-]^+$ (the dominant solution species formed when **2**·2HCl is dissolved in methanol[13]) is displaced by an added phosphate anion. Thus, titrations involving the addition of sapphyrin free-base **2** to either phosphoric acid, phenyl phosphoric acid, or phenylphosphonic acid in methanol-d_4 gave rise to binding isotherms that were consistent with binding in accord with eq 4 (K_{22}) early on in the titration process (i.e., at phosphate-to-sapphyrin molar ratios >5) and eq 5 (K_{11}) at later junctures where the phosphate-to-sapphyrin molar ratios are substantially decreased. Qualitatively similar behavior was also observed when the titrations were run using **2**·HCl as the added sapphyrin species: At low phosphate-to-sapphyrin ratios, K_{11} binding behavior consistent with phosphate binding according to eq 5 or facile phosphate-for-chloride substitution behavior K_{1ex} (eq 7 → eq 5 in the limit where K_{11} for chloride anion is infinitely small), is observed, while at higher ratios (i.e., early on in the titration), K_{22}-like binding processes, associated with $[2H\cdot\mathbf{2}]^{2+}\cdot Cl^-\cdot$[phosphate]$^-$ and/or $[2H\cdot\mathbf{2}]^{2+}\cdot 2$[phosphate]$^-$ formation, are observed. By contrast, addition of **2**·2HCl to methanol-d_4 solutions of phosphoric acid or phenylphosphonic acid gives rise to titration profiles (i.e., binding isotherms) that, except for data points recorded at very high phosphate-to-sapphyrin salt ratios (where K_{22}-like binding processes could still be important), are best interpreted in terms of phosphate-for-chloride exchange as defined by eq 6 and K_{2ex}. Simple multiplication by 100 (the value of K_{21} for the formation of $[2H\cdot\mathbf{2}]^{2+}\cdot Cl^-$ in methanol) thus gave K_{21} for the phosphate species in question (cf. Table 3).[31]

In the case of phenylphosphonic acid studied in methanol-d_4, association constants were also obtained from ^{1}H NMR

(29) (a) Aoyama, Y.; Nonaka, S.; Motomura, T.; Toi, H.; Ogoshi, H. *Chem. Lett.* **1991**, 1241−1244. (b) Marzilli, L. G.; Banville, D. L.; Zon, G.; Wilson, W. D. *J. Am. Chem. Soc.* **1986**, *108*, 4188−4192.

(30) These large shifts do not appear to be due to simple deprotonation effects as confirmed by the fact that monobasic potassium and monobasic triethylammonium salts of phenylphosphonic acid display ^{31}P NMR resonances in methanol-d_4 that are displaced upfield by only 3.23 and 2.93 ppm, respectively, relative to those of the free-acid form.

(31) Many other titrations were also carried out using a range of other phosphate−sapphyrin mixtures with both compounds being in various protonation states, e.g., free-base, mono- and diprotonated sapphyrin, free-acid, mono- and dibasic phosphate acids. See Supporting Information.

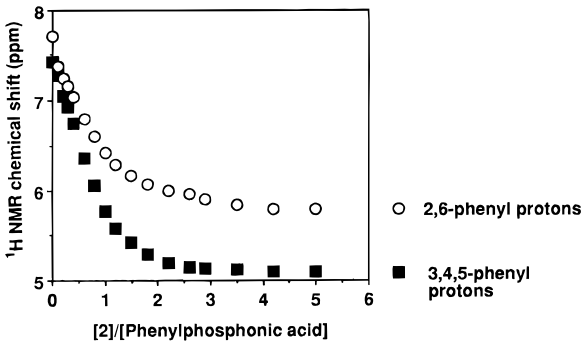

Figure 9. Changes in phenyl proton NMR signals as phenylphosphoric acid is titrated with increasing quantitities of sapphyrin **2** in methanol-d_4. For experimental conditions, see text.

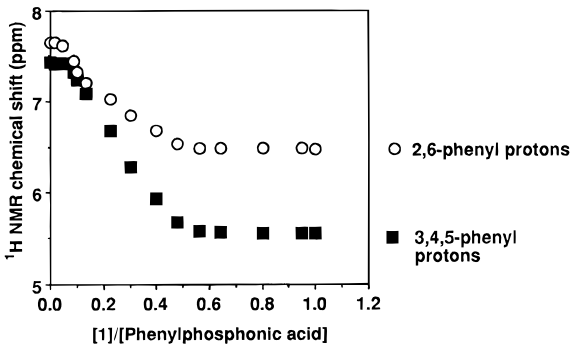

Figure 10. Changes in phenyl proton NMR signals as phenylphosphoric acid is titrated with increasing quantitities of sapphyrin **1**. For experimental conditions, see text.

titrations (Figure 9) with the resulting values being likewise included in Table 3. Here, advantage was taken of the fact that large upfield shifts in the phenyl proton resonances (a pair of 2H and 3H multiplets) are observed as the titration was allowed to progress. These shifts, as above, are ascribed to the sapphyrin ring current and the influence it exerts on the presumably $\pi-\pi$ stacked phenyl ring above it. Thus, these ^{1}H NMR findings for phenylphosphonate in methanol-d_4 are very much consistent with the solid-state structure obtained for the corresponding phenyl phosphate complex (cf. Figure 4).

^{1}H NMR analyses were also used to check the protonation state of sapphyrin prior to and during the ^{31}P NMR titrations. This was done by monitoring the chemical shift of the sapphyrin methine protons at different sapphyrin-to-phosphate ratios and comparing the values with those obtained under well-defined control conditions. In methanol-d_4, the free-base sapphyrin **2** yielded a chemical shift for the methine protons of 10.52 and 10.89 ppm. For the corresponding monoprotonated phosphate-bound species, the values were 11.58 and 11.65 ppm, and for the diprotonated sapphyrin species the value was 11.84 ppm (only one signal observed). The same trend for the change of these methine protons could be followed in chloroform-*d* and chloroform-*d*/methanol-d_4 mixtures (4:1). Recorded chemical shift values in pure chloroform-*d* are as follows: free-base sapphyrin **2**, 10.62 and 10.82 ppm; monoprotonated phosphate-bound species, 11.34 and 11.43; diprotonated diphosphate bound species, 11.80 and 11.85. For these control studies, the phosphate or phosphonate substrate in question was used in its monoanionic form (i.e., $H_2PO_4^-$, PhP(O)(OH)O$^-$); these species were generated either by addition of the appropriate amount of the sapphyrin free-base or by direct use of the phosphate monoanionic salts followed by titration with the monoprotonated HCl salt of sapphyrin.

Some ^{1}H NMR experiments were also carried out in D_2O using the water-soluble sapphyrin system **1**. As was true in the case of the ^{31}P NMR analyses, however, poor solubility and a tendency toward aggregation precluded a quantitative NMR study. However, at low pH values and using high phenylphosphonic acid-to-sapphyrin ratios (i.e., where the diprotonated form of sapphyrin was expected to be dominant), it proved possible to follow the changes in chemical shift (to higher field) for the phenyl protons as a function of increasing sapphyrin concentration.[32] A typical binding profile generated in this way is shown in Figure 10. It displays a clear "break" at the point where 0.5 molar equiv of sapphyrin free-base **1** had been added to a solution of phenylphosphonic acid in D_2O (initial pH 2.0). This is consistent with the formation of a 2:1 phosphate-to-sapphyrin complex as would be expected under conditions such as these where the sapphyrin nucleus is not only diprotonated but also exposed to a high relative phosphate concentration.

As implied above, anion competition experiments could be used to determine relative association constants for the phosphorylated species relative to Cl$^-$, F$^-$, and other anions. This was done most easily by monitoring the relative shift in the ^{31}P NMR resonances of 1:1 sapphyrin−phosphate or 1:1 sapphyrin−phenylphosphonate solutions (molar ratios) in methanol-d_4 in the presence and absence of 1 molar equiv of the competing anion (X$^-$) being examined (Table 4). Since K_{21} values for $H_2PO_4^-$ and PhP(O)(OH)O$^-$ binding could be independently determined from the full chloride anion exchange "titrations" described above, these measurements provide a direct, but relative, measure of the association constants for the complexation of other anions, X$^-$. Relevant results and control experiments are summarized in Table 4.

In methanol-d_4, F$^-$ was found to be the most strongly bound of all anions studied. In fact, it was so strongly bound that an accurate K_{rel} could not be readily obtained using the above competition approach ($K_{rel} \leq 0.1$). This is not surprising, given the association constants, K_{21}, found for the binding of Cl$^-$ (10^2 M^{-1}), dihydrogen phosphate ($10^{4.2}$ M^{-1}), and F$^-$ (10^5 M^{-1}) to diprotonated sapphyrin. What was less expected, however, was the finding that no other anion type studied (*viz.* nitrate, carboxylate, bromide, and perchlorate) was bound to diprotonated sapphyrin with an affinity approaching that observed for the complexation of dihydrogen phosphate or monobasic phenylphosphonate. Nonetheless, these results provide an indication that the diprotonated forms of sapphyrin are not only *effective* but also reasonably *selective* phosphate anion receptors.

Visible Spectroscopy. Visible spectroscopy (vis) provided another means of monitoring the phosphate-to-sapphyrin complexation processes.[17] In methanol, the Soret band of sapphyrin free-base **2** undergoes a ca. 5-nm shift to the blue as a 1:2 diprotonated sapphyrin−phosphate complex is formed. Discernible changes in the Q-band region, reflecting changes in the symmetry of the molecule, were also observed: Whereas free-base sapphyrin **2** displays 3 Q bands, the 1:1 sapphyrin−phosphate complexes, which are of lower overall symmetry, are characterized by 4 Q bands. On the other hand, the 1:2 diprotonated sapphyrin−phosphate complexes, which are of higher molecular symmetry, are generally found to display but 2 Q-type bands. These complexities limited the use of visible spectroscopy in carrying out quantitative association constant determinations. In the case of phenylphosphonic acid, however, observed changes in the optical signature of the Soret band as a function of concentration could be used to derive a K_{22} value (cf. Figure 9 in supporting information). Here, data reduction was effected in accord with standard procedures (c.f. Experi-

(32) In these experiments, the sapphyrin methine protons could also be monitored. As expected, these sets of signals also moved to higher field as complexation was allowed to occur.

Table 4. Relative Binding Affinities (K_{rel}) of Sapphyrin for Various Anions, X^-, in Methanol-d_4 As Determined from Competition Experiments Involving Displacement of Initially-Chelated Monobasic Phosphate ($H_2PO_4^-$) or Phenylphosphonate and Monitored by ^{31}P NMR Spectroscopy

phosphate[a] species	macrocycle	anion X^-	^{31}P chem shift δ (ppm)[b]	$\Delta\delta$ (ppm)	K_{rel}
phosphoric acid	none	none	1.67		
phosphate[c]	none	none	1.99		
phosphate	**2**	none	−5.38	7.37	
phosphate	**2**	Br^-	−5.00	0.38	19.4
phosphate	**2**	Cl^-	−5.19	0.19	38.8
phosphate	**2**	F^-	1.74	7.12	<0.1
phosphate	**2**	AcO^-	−5.15	0.23	32.0
phosphate	**2**	$CF_3CO_2^-$	−5.16	0.22	33.5
phosphate	**2**	$C_6H_5CO_2^-$	−3.48	1.90	3.9
phosphate	**2**	NO_3^-	−4.78	0.60	11.8
phosphate	**2**	ClO_4^-	−4.60	0.78	9.1
phenylphosphonic acid	none	none	16.77		
phenylphosphonate[c]	none	none	13.84		
phenylphosphonate	**2**	none	7.68	6.16	
phenylphosphonate	**2**	F^-	13.50	5.82	<0.1
phenylphosphonate	**2**	Cl^-	7.79	0.09	68.4

[a] All spectra were recorded at a 1:1 phosphate-to-sapphyrin **2** molar ratio and a phosphate-to-sapphyrin **2**-to-X^- anion molar ratio of 1:1:1 unless noted otherwise. The concentration of all components was 5 mM. [b] The external chemical shift reference is phoshoric acid and was set to 0.0 ppm. [c] As the mono triethylammonium (or tetrabutylammonium) salts generated in situ. [d] $K_{rel} < 0.1$; an accurate value could not be obtained by competition as full saturation is already obtained in the presence of 1 equiv of F^-. Proof of this came from a complete titration curve; see the complete titration curve in Figure 3 of the supporting information.

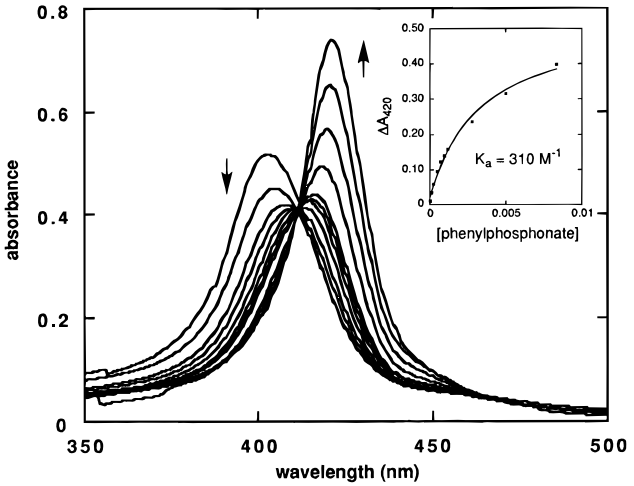

Figure 11. Overlay of the visible spectra of a 6 μM solution of sapphyrin **1** recorded in the presence of increasing concentrations of phenylphosphonate in 10 mM bis-Tris buffer, pH 6.1. The insert shows a plot of the absorbance at 420 nm of sapphyrin **1** as a function of added phenylphosphonate in 10 mM bis-Tris buffer, pH 6.1. Also shown is an interpolative fit (—) for the apparent K_a of the dimeric form of sapphyrin **1** binding to phenylphosphonate; this value is 310 M^{-1}.

mental Section). The value so obtained is included in Table 3 along with that derived from the NMR titrations alluded to above.

Vis titrations could be carried out in 10 mM aqueous bis-Tris buffer (pH 6.1) by monitoring the spectral changes associated with sapphyrin deaggregation. Specifically, the water-soluble sapphyrin **1** when dissolved in such an aqueous medium (concontration 6×10^{-6} M) showed an absorbance band at $\lambda_{max} \approx 400$ nm, corresponding to the aggregated form. Adding phenylphosphonate or phenyl phosphate (0−2000 equiv) gave rise to *one* new band at $\lambda_{max} \approx 420$ nm, corresponding to a phosphate-bound dimeric sapphyrin species (Figure 11). Adding increasing quantities (0−2000 equiv) of AMP, GMP, CMP, or UMP to identical initial sapphyrin solutions gave rise to *two* new bands, at $\lambda_{max} \approx 420$ and 445−450 nm, corresponding to phosphate-bound dimeric and monomeric sapphyrin species, respectively. The band at ≈450 nm grew in more noticeably in the case of AMP and GMP than it did when CMP and UMP were used. Adding diphenyl phosphate led to a clear diminution of the initial absorbance band at $\lambda_{max} \approx 400$ nm with a concomitant growing in of two new bands at ≈427 and 450 nm. Although reasonable isobestic behavior was observed in this titration, the nature of the species giving rise to this spectral signature is still uncertain; they are tentatively assigned, however, as being phosphate-bound dimeric and monomeric sapphyrin species in analogy to the above. Titrations involving sapphyrin **1** and phosphoric acid, on the other hand, did not show a clean isobestic point. This precluded attempts to determine effective binding constants for this particular substrate−receptor pair.

The apparent association constants for the formation of a phosphate-bound dimeric sapphyrin species starting from the aggregated form of **1** were calculated by fitting the binding curve of absorbance at 420 nm as a function of change in phosphate anion concentration as detailed in the Experimental Section. Such analyses yielded binding constants of 310, 280, and 300 M^{-1} for phenylphosphonate, diphenyl phosphate, and phenyl phosphate, respectively; 160 and 190 M^{-1} for AMP and GMP, respectively; and 130 and 110 M^{-1} for CMP and UMP, respectively. These binding constants are analogous to K_{11} of eq 5 and thus somewhat comparable to the equilibrium constants measured in methanol. However, they refer to the formation of a phosphate-bound sapphyrin *dimer*, not inner-sphere 1:1 complexes involving a sapphyrin *monomer*. Further discussion of this point, along with experimental justifications for the spectral assignment, is provided in the ensuing paper.

pK_a Determinations. In prior work,[15,16] approximations for the acid disassociation constants of eqs 1 and 2 were made by monitoring changes in the optical spectrum of sapphyrin **3** in organic solution (i.e., dichloromethane) as a function of a change in pH of an aqueous solution with which it was in contact. Effective "kinetic pK_a" values were also determined by monitor-

(33) While the measured values are precise, relatively large error bars are placed on these values because ionic strength was not maintained during the course of the pH titration. Sapphyrin **1** is insoluble under the constant ionic strength requirements (typically 100 mM $NaClO_4$) used for standard pK_a determinations. A nonionic detergent was employed in this study to avoid difficulties involving the precipitation of the neutral macrocycle at high pH. Interestingly, the monocation of sapphyrin **1** is clearly stable under these conditions, a phenomenon that, to the best of our knowledge, has not been observable with octaalkyl porphyrins in the presence of nonionic detergents. See: Phillips, J. N. In *Current Trends in Heterocyclic Chemistry*; Alberts, A., Badger, G. M., Shoppee, C. W., Eds.; Academic Press, Inc.: New York, 1958; pp 30−39.

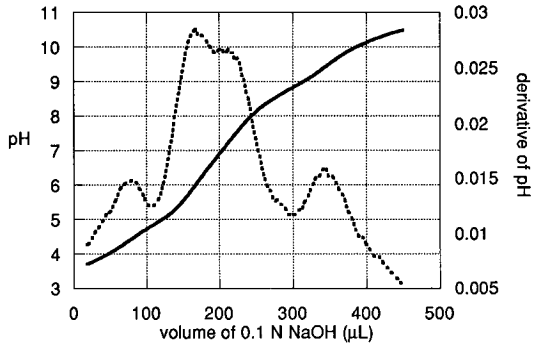

Figure 12. The pH titration curve that results when **1**·2HCl in a 2.5% (w/w) aqueous solution of Tween 20 is titrated with NaOH (—). Also shown is the first derivative of this curve (- - -) with minima that correspond to pK_a values of 4.8 and 8.8. For experimental conditions, see text.

ing the rate of *p*-toluenesulfonate anion transport through a dichloromethane barrier in our now-standard, Pressman-type Aq I−CH_2Cl_2−Aq II model membrane system.[14−16a,b] Both of these studies served to show that, in the absence of any particularly strong chelating anion, sapphyrin **1** is effectively diprotonated (>95%) below ca. pH 3.0 and monoprotonated (>95%) for $4 \leq$ pH ≤ 9.

With the advent of the new water-soluble sapphyrin (**1**) described in this report, it became possible to measure the pK_a values for sapphyrin more directly. A pH titration carried out in 2.5% Tween 20 using the well-characterized bishydrochloride salt of tetrahydroxysapphyrin **1** gave values for pK_{a1} and pK_{a2}, corresponding to the loss of one and two protons from the starting diprotonated form, of 4.8 ± 0.2 and 8.8 ± 0.2, respectively.[33] It is gratifying that values obtained from this measurement are largely consistent with earlier measurements: Although doubly protonated at low pH, these measurements, taken together, indicate that sapphyrin remains monocationic at and near neutral pH. By comparison, octaalkylporphyrins, with typical pK_{a2} values between 3.0 and 5.8,[10] are known to be neutral entities at pH 7.0. Unfortunately, efforts to obtain pK_a values for porphyrin **4** failed due to low solubility in aqueous solutions of neutral or basic pH.

Conclusion

Taken together, the present results support the notion that the protonated forms of sapphyrin function as highly effective phosphate anion receptors. The observed complexes, which can be obtained using either the mono- or diprotonated forms of sapphyrin, are thought to arise as the result of a propitious combination of oriented hydrogen-bonding interactions and favorable electrostatic attractions between the negatively charged phosphate substrate and the positively charged mono- or diprotonated sapphyrin cores. Thus, the basic structural features of sapphyrin (large core size, basic nitrogens, neutral and protonated NH residues, etc.) are thought to be responsible for its distinct phosphate recognition behavior. Because of this link between structure and function, we are presently trying to ascertain which of these features is most important in terms of mediating phosphate complex formation. Specifically, we are trying to determine the extent to which modifications of the basic sapphyrin skeleton (e.g., O for NH replacement) are reflected in differences in phosphate anion binding affinity. Also, as detailed in the ensuing paper, we have for some time been interested in understanding how protonated sapphyrins and their derivatives might interact with other, more biologically relevant phosphate-containing substrates.

Acknowledgment. We thank Ms. Petra Sansom for her help in carrying out the visible titration studies and for her and Mr. Michael Choe's assistance with manuscript preparation. This work was supported by grants from the Texas Advanced Technology Program (Grant No. 3658-280) and the NIH (Grant No. AI 33577). Partial support from Pharmacyclics, Inc. (to J.L.S.) and the Howard Hughes Medical Institute (to J.L.S. and V.K.) is also gratefully acknowledged.

Supporting Information Available: Figures, tables, and text giving summaries of further optical and NMR analyses and full analysis of the X-ray diffraction data for inner-sphere complexes $[2H\cdot\mathbf{3}]^{2+}\cdot 2[C_6H_5OP(O)(OH)O]^-$, $[2H\cdot\mathbf{2}]^{2+}\cdot 2[(C_6H_5O)_2P(O)O]^-$, $[[2H\cdot\mathbf{2}]^{2+}\cdot[H_2PO_4]^-]^+$, and $[2H\cdot\mathbf{2}]^{2+}\cdot[HPO_4]^{2-}$ (152 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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