Sapphyrins: Versatile Anion Binding Agents

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ABSTRACT

Sapphyrin was the first expanded porphyrin to be reported in the literature and remains among the most extensively studied. Much of the interest in this macrocycle reflects its ability to bind anions, a phenomenon that has been examined in solution and in the solid state by a wide range of experimental techniques. In this Account, we summarize these studies while also outlining strategies that may be used to synthesize sapphyrins.

Porphyrins and related tetrapyrrolic macrocycles serve a variety of critical roles in living systems and are present in such functionally disparate systems as photosynthetic reaction centers, coenzyme B_{12}, hemoglobin, myoglobin, cytochromes, peroxidases, and catalyases to name but a few. Consequently, porphyrins remain among the most widely studied of all macrocyclic systems.[1−4] In recent years, the scope of porphyrin-related research has broadened with the emergence of the "expanded porphyrins", polypyrrolic macrocycles that contain more coordinating heteroatoms, larger central binding cores, and/or more extensive π-electron conjugation pathways than the porphyrins.[4−6] The study of expanded porphyrins is inspired by the potential to combine some of the advantageous features of porphyrins (e.g., light absorption, singlet oxygen generation, etc.) with unique characteristics that the porphyrins do not possess, such as anion binding.[4−6]

Many expanded porphyrins are known today, with new ones being reported with increasing regularity.[4−6] Nonetheless, sapphyrin, the first expanded porphyrin system ever to be reported,[7−9] remains one of the most interesting. As shown schematically in Figure 1, the core of sapphyrin is expanded relative to that of porphyrin in that it contains an additional pyrrole, inserted between a *meso*-carbon and an α-pyrrolic position. Like corrole, sapphyrin contains a direct α−α pyrrole connection not present in porphyrin. Despite this structural elegance, for many years the sapphyrins were thought of as mere chemical curiosities, i.e., difficult-to-access polypyrrolic macrocycles that lacked the versatile metal-ligation properties of the porphyrins.[10] In 1990, our group reported a startling finding, namely that diprotonated sapphyrin forms a complex with fluoride anion in the solid state.[11] This led us to propose that sapphyrins might act as versatile anion binding agents. This conception, which was without precedent in the porphyrin literature, provided an impetus to reinvestigate the chemistry of sapphyrins. In a more general sense, it also helped fuel the current high level of interest in expanded porphyrin chemistry. The purpose of this Account is thus to chronicle the exploration of the anion binding properties of sapphyrin as well as to provide an overview of the various synthetic methods that may be used to prepare this most quintessential of expanded porphyrins. Not discussed in this Account is the limited metalation chemistry of sapphyrins. This subject has been treated elsewhere.[4]

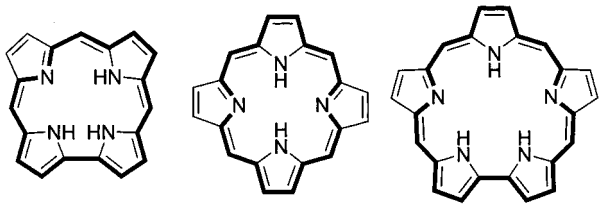

FIGURE 1. A comparison of the core structures of corrole, porphyrin, and sapphyrin, with the shortest π-electron conjugation pathway of each macrocycle shown in bold.

Discovery and Synthesis

In the early 1960s, work directed toward the synthesis of vitamin B_{12} by the Woodward group led to a serendipitous discovery.[7] Treatment of a linear tetrapyrrole (**8**, Scheme 2) with formic and hydrobromic acids followed by oxidation with iodide gave rise not to the desired corrole, but instead to a pentapyrrolic byproduct. They assigned the trivial name "sapphyrin" to this compound, in light of its brilliant blue color in the solid state. This product later proved to contain the pentapyrrolic core shown in Figure 1, with its 22 π-electron aromatic periphery highlighted in bold.[8−10]

Broadhurst, Grigg, and Johnson published the first rational synthesis of a sapphyrin; specifically, the condensation of tripyrrane **1** with bisformyl bifuran **2**, followed by oxidation, afforded dioxasapphyrin **3**, as shown in Scheme 1.[8] This type of method is referred to as a [3 + 2] approach for the number of pyrrole-like units in each precursor. After their initial report, these researchers as well as, independently, colleagues and co-workers of Woodward used an analogous approach to generate all-aza sapphyrins such as **4** in yields up to 71% for the key sapphyrin-forming step.[9,10] A generalized [3 + 2] method

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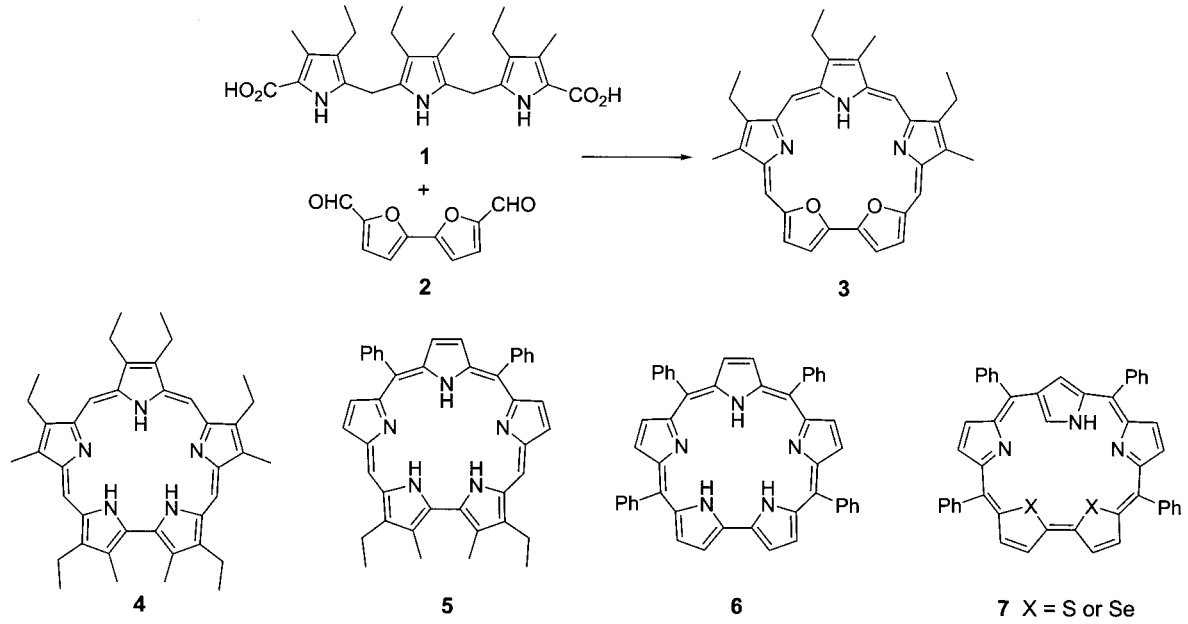
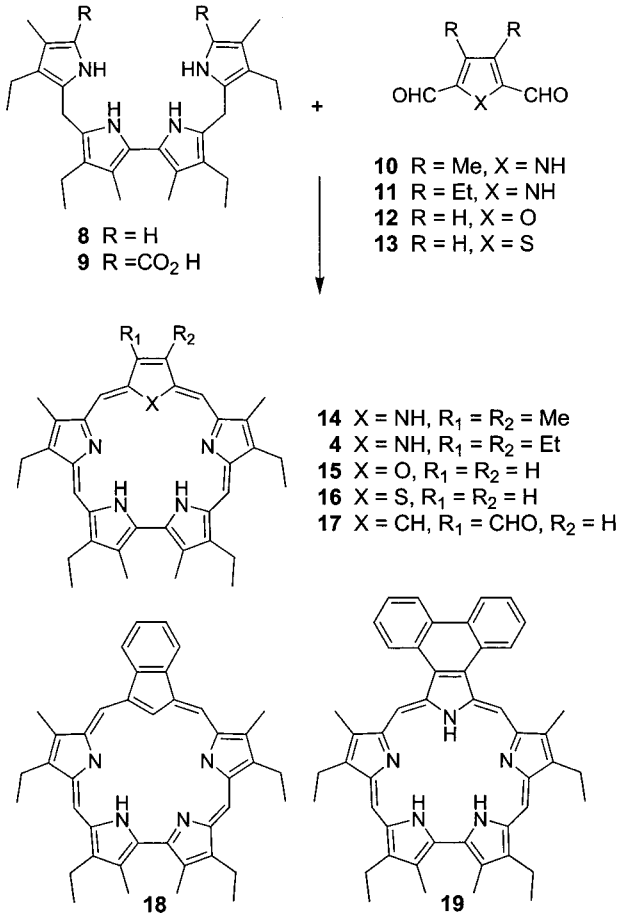
Scheme 1



Scheme 2



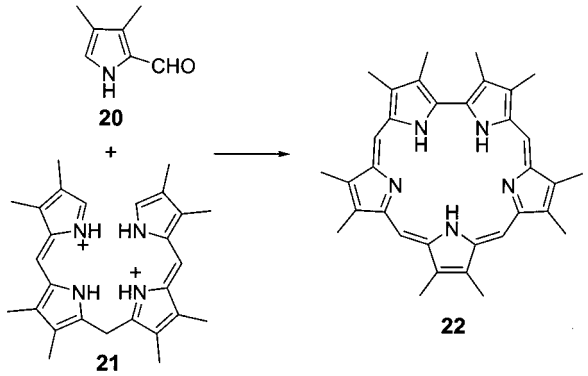
has also been employed by Dolphin et al. to obtain *meso*-diphenyl-substituted sapphyrins, such as **5**, as well as tetraphenylsapphyrin **6**.[12] Chandrashekar and co-workers have reported the use of an acid-catalyzed [3 + 2] condensation in the syntheses of a wide variety of tetraphenyl heterosapphyrins (sapphyrin in which core nitrogens have been replaced with other heteroatoms such as O, S, and Se).[13−15] Quite recently, Furuta and Chandrashekar have used a [3 + 2] approach to generate a so-called inverted, N-confused sapphyrin **7**.[16]

A [4 + 1] synthesis, shown in Scheme 2, was also reported by Woodward and co-workers. In this synthesis, the tetrapyrrole **9** was combined with the bisformyl pyrrole **10** to produce, after oxidation, sapphyrin **14**.[10] The higher yields of the [3 + 2] reactions, coupled later with a streamlining of the requisite precursor syntheses,[11] made them the method of choice for some time. As a result, generalized [4 + 1] strategies have been largely overlooked until quite recently. In 1999, Richter and Lash reported that the yield of sapphyrins such as **4** via this [4 + 1] method can be improved by utilizing dilute aqueous ferric chloride as the oxidizing agent.[17] Richter and Lash have also reported the use of this methodology to synthesize heterosapphyrins **15** and **16**, as well as carbosapphyrins such as **17** and **18**, in which a pyrrolic NH center has been replaced with a CH moiety, and novel sapphyrins such as **18** and **19**, which contain additional aromatic rings fused to the β-pyrrolic positions of one pyrrole.[17]

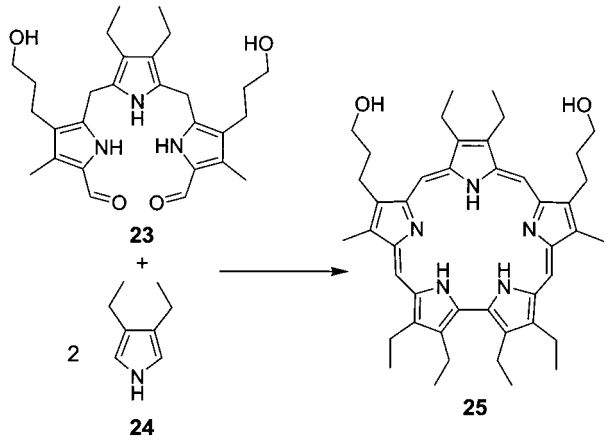
An alternate [4 + 1] approach, shown in Scheme 3, was recently reported by Paolesse and Smith.[18] The tetrapyrrolic precursor in this case was an a,c-biladiene (**21**) which was reacted with formylpyrrole **20**. This reaction is presumed to proceed through a linear pentapyrrolic intermediate which cyclizes via formation of the direct pyrrole−pyrrole link to afford sapphyrin **22**.

The latter report provides a reminder that direct pyrrole−pyrrole bonds can be formed during the macrocyclization reactions used to prepare expanded porphyrins. Recognizing this, we developed a [3 + 1 + 1] condensation procedure, wherein sapphyrins such as **25** are prepared by condensing a bisformyl tripyrrane such as **23** with two molar equivalents of a bis-α-free pyrrole (e.g., **24**), as illustrated in Scheme 4.[19] This synthesis is

Scheme 3



Scheme 4



more efficient for β-alkyl-substituted sapphyrins than the [3 + 2] approach in terms of both overall yield and total number of steps, for the simple reason that the key bipyrrole unit is formed in situ rather than as a precursor.[19]

Sapphyrin-forming reactions in which four or five pyrrole−pyrrole connections in sapphyrin are formed in a single reaction vessel without additional workup or manipulations have come to be known as "one-pot" reactions. The Woodward group observed early on that sapphyrin **22** was formed in small amounts when 3,4-dimethylpyrrole was condensed with 2,5-diformyl-3,4-dimethylpyrrole under conditions of acid catalysis.[10] While this observation marked the first one-pot, [1 + 1 + 1 + 1 + 1] synthesis of sapphyrin, this approach did not become fully appreciated 1995,[20] when Latos-Grazynski et al. reported the isolation of tetraphenylsapphyrin **6** from a Rothemund-type reaction between unsubstituted pyrrole and benzaldehyde. Around the same time, we found that reaction of a bisformyl bipyrrole with three equivalents of pyrrole and two equivalents of benzaldehyde afforded, after oxidation, the corresponding diaryl sapphyrin (e.g., **5**) in low yield (ca. 10%).[21] The groups of Latos-Grazynski[22] and Chandrashekar[23] have used one-pot reactions to prepare a variety of tetraphenyl heterosapphyrins. Although these various "one-pot" syntheses are often inefficient and tend to produce many macrocyclic and linear byproducts, they offer the advantage of requiring only the simplest of monopyrrolic precursors.

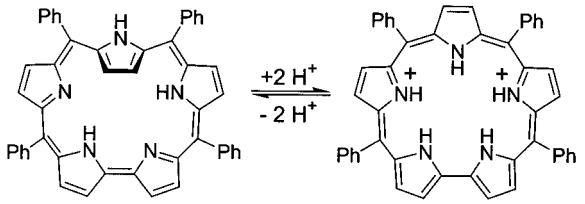


FIGURE 2. Equilibrium between the inverted and planar conformations of tetraphenyl sapphyrin **6**.

Table 1. Select Spectroscopic Data for Diethylhexamethylcorrole,[2,4] Octamethylporphyrin,[10] and Decamethylsapphyrin[10]

	corrole	porphyrin	sapphyrin
Soret λ_{max}, nm (log ϵ)	413 (5.16)	414 (5.42)	456 (5.77)
^{1}H NMR δ, ppm			
NH	−3.48	−4.82	−5.46, −5.00, −4.84
meso-H	8.82, 9.12	10.98	11.51, 11.70

Spectroscopic Properties

The shortest π-conjugation pathway around the core of sapphyrin, shown in bold in Figure 1, contains 22 π-electrons. This makes sapphyrins examples of heteroannulenes that are one aromatic "size" larger than the porphyrins and corroles, both of which contain 18 π-electron peripheries. The Soret-like absorbance maxima and key ^{1}H NMR shifts of a representative β-alkyl, *meso*-free corrole, porphyrin, and sapphyrin are summarized in Table 1. In a general sense, the spectroscopic properties of sapphyrin are consistent with their being considered as prototypical expanded porphyrins. The Soret-like absorbances of sapphyrins are relatively red-shifted and generally more intense than those of corrole or porphyrin. Additionally, slightly larger shielding and deshielding effects are observed for the pyrrolic NH and *meso*-H signals in the ^{1}H NMR spectra of sapphyrins.

NMR spectroscopic studies by the Latos-Grazynski group led to an appreciation that the free-base form of tetraphenyl sapphyrin **6** adopts a conformation wherein one pyrrole is inverted such that the NH atoms face outward from the center of the core.[20] They demonstrated that protonation of **6** induces a rearrangement to the typical planar conformation in which all the nitrogens are oriented inward, as depicted in Figure 2. The planar, protonated form was later found to exist in equilibrium with an inverted, protonated form.[24] As a general rule, inverted conformations are not seen in the case of β-alkyl-substituted sapphyrins.[25]

Anion Binding

As noted in the introduction to this Account, the first crystallographic analysis of sapphyrin, carried out in 1990,[11] led to the proposal that protonated sapphyrins might function as effective anion receptors. While now a major research direction in the area of polypyrrolic macrocyclic chemistry, the evolution of this idea was actually fortuitous. In fact, the X-ray structural analysis carried out in 1990 involved a crystal initially thought to be the bis-PF_6^- salt of the doubly protonated form of

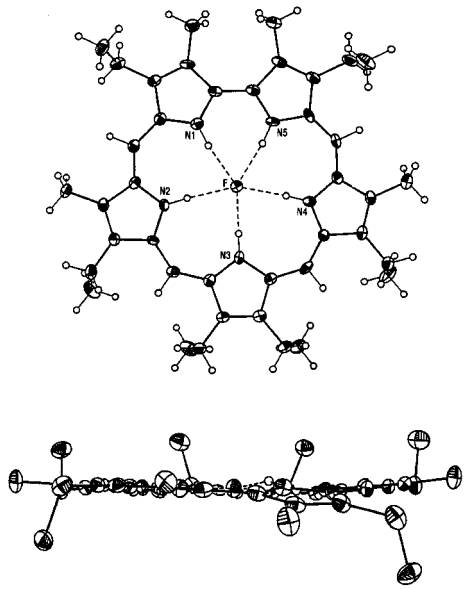


FIGURE 3. Two views of the $[H_2\mathbf{4}\cdot F]^+$ cation. One equivalent of PF_6^- (not shown) was found in the crystal lattice. Reproduced with permission from ref 26. Copyright 1992 American Chemical Society.

sapphyrin **4** ($H_2\mathbf{4}^{2+}$). However, as can be seen by inspection of the structure actually resolved (cf., Figure 3), a fluoride atom was found inside the sapphyrin core. Presumably, the bound fluoride anion had its origins either as an impurity in the PF_6^- or as the result of a sapphyrin-induced $PF_6^- \rightleftarrows PF_5 + F^-$ disassociation process. In the structure itself, the nitrogens and the fluoride were found to be nearly coplanar, with a mean deviation of only 0.03 Å, while the bound fluoride center was found to be within hydrogen-bonding distance of all five nitrogens.[11]

The above discovery led naturally to the question of whether other anions could coordinate in this manner. The results of a single-crystal X-ray structural analysis of the dihydrochloride salt of **4**, carried out in 1992, are shown in Figure 4.[26] The two chloride anions are apparently too large to be accommodated within the sapphyrin plane. They are thus bound to opposite faces of the diprotonated sapphyrin macrocycle via hydrogen bonds. In dichloromethane solution, the visual absorbance maxima of **4**·2HF (446 nm) was found to be blue-shifted relative to those of **4**·2HCl and **4**·2HBr (456 and 458 nm, respectively). A somewhat different redox profile was also recorded for the fluoride complex, and taken together these observations are consistent with a binding motif for fluoride anion (encapsulation) that is different from that for the larger halide anions.

Compared to what is seen for dichloromethane solutions of **4**·2HF, the emission of solutions of both **4**·2HCl and **4**·2HBr were found to be extensively quenched. The relative absence of quenching effects in the fluoride complex was consistent with the fluoride anion interacting strongly with the core NHs of sapphyrin, thus reducing the effectiveness of this vibrational energy sink.[26] Two discrete fluorescence lifetimes were observed for dichloromethane solutions of **4**·2HCl and **4**·2HBr, in contrast to the fluoride complex, which exhibited only one

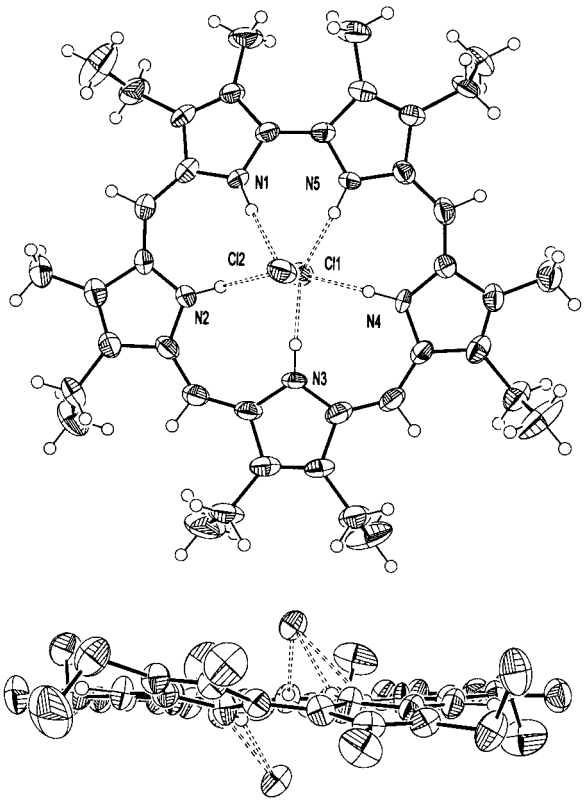


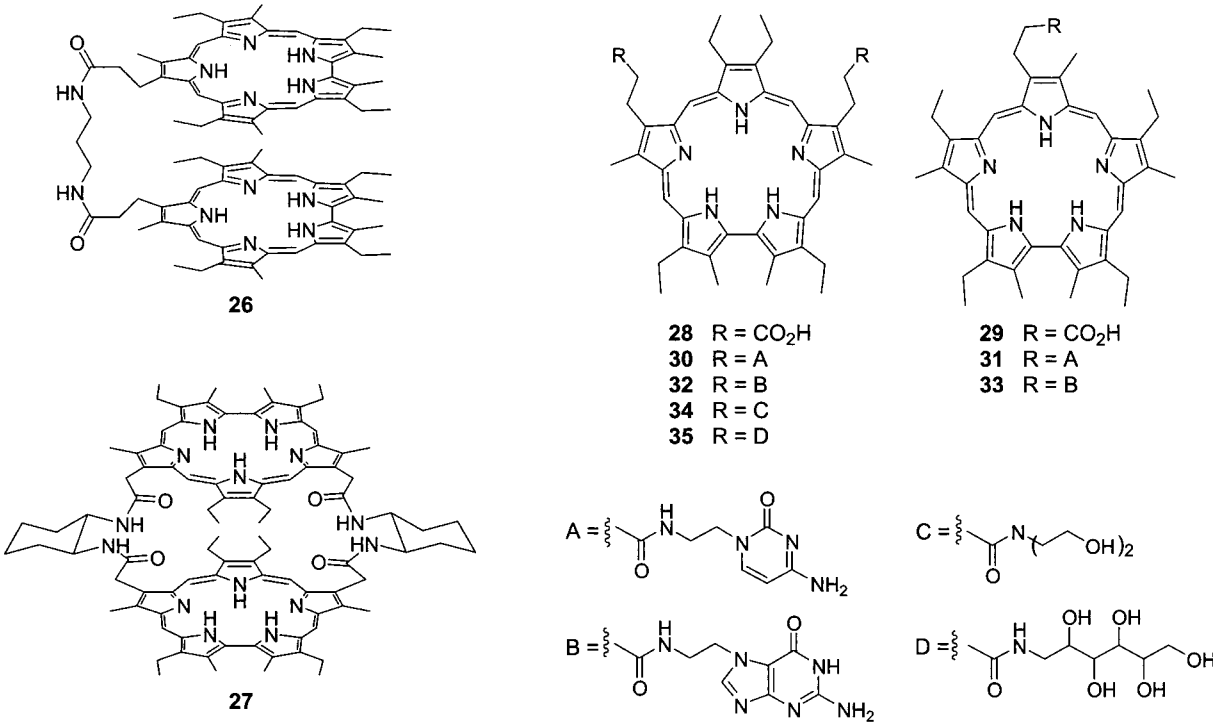
FIGURE 4. Two views of the molecular structure of the **4**·2HCl complex. Hydrogen-bonding interactions indicated with dashed lines. This figure was generated from results first published in ref 26.

Table 2. Association Constants for Protonated Sapphyrins with Various Anions

sapphyrin (method)[ref]	anion	K (M^{-1})
Solvent: H_2O, pH 6.1		
34 (UV−vis)[40]	$C_6H_5PO_3^{2-}$	310
Solvent: CH_3OH		
4 (UV−vis)[26]	F^-	2.8×10^5
25 (^{31}P NMR)[29]	$C_6H_5PO_3^{2-}$	1.8×10^4
	H_3PO_4	1.3×10^4
31 (UV−vis)[35]	2′GMP	2.2×10^4
	5′GMP	8100
	5′AMP	1700
	5′CMP	880
4 (UV−vis)[26]	Cl^-	100
	Br^-	<100
26 (^{2}H NMR)[30]	terephthalate	4600
Solvent: 5% CH_3OH in CH_2Cl_2		
27 (UV−vis)[31]	*N*-CBZ-L-ASP	1.6×10^4
	N-CBZ-D-ASP	9700
	N-CBZ-L-GLU	3800
	N-CBZ-D-GLU	1.6×10^4
Solvent: CH_2Cl_2		
4 (fluorescence lifetime)[26]	F^-	$>10^8$
	Cl^-	1.8×10^7
	Br^-	1.5×10^6

lifetime. Association constants for $H_2\mathbf{4}^{2+}$ and chloride (1.8×10^7 M^{-1}) and bromide (1.5×10^6 M^{-1}) anions were calculated from these data (cf. Table 2). The association between $H_2\mathbf{4}^{2+}$ and fluoride was found to be too strong to measure accurately in dichloromethane, though a lower limit (10^8 M^{-1}) was established. Association constants for $H_2\mathbf{4}^{2+}$ in methanol with fluoride, chloride, and bromide anions were determined from fluorescence titration ex-

Chart 1



periments and found to be 2.8×10^5, 10^2, and $<10^2$ M^{-1}, respectively.[26] This clear selectivity of 3 orders of magnitude for fluoride anion over chloride or bromide anion was completely unexpected on the basis of a normal Hofmeister progression.[27] On the other hand, it was considered fully consistent with the special, stabilizing in-plane hydrogen motif seen in the solid-state structure of $[H_2\mathbf{4}^{2+}\cdot F^-]\cdot PF_6^-$, being largely, or even completely, retained in methanol solution.[26]

Further insights into the nature of fluoride binding by the protonated forms of sapphyrin were obtained by carrying out anion transport experiments using a standard Pressman-type U-tube membrane model. The presence of sapphyrin **4** was found to enhance the transport of the fluoride anion through the organic phase of this model over a wide pH range. At neutral pH, where it was first inferred[28] and then established (vide infra) that sapphyrin is monoprotonated,[29] the enhancement was nearly 2 orders of magnitude relative to the background rate. In comparison, the presence of octaethylporphyrin in the organic phase improved the transport rate by only a factor of 3.[28]

Several covalently linked sapphyrin dimers, including **26** and **27** (Chart 1), were prepared in the hope of creating a ditopic receptor for dianions such as dicarboxylates.[30,31] These covalent dimers were found to display a unique feature in their visible absorbance spectra, namely two Soret-like maxima in both methanol (ca. 422 and 441 nm) and dichloromethane (ca. 426 and 450 nm). The origin of these two bands was ascribed to the existence of two conformational states, shown schematically in Figure 5, the so-called (a) "open" and (b) "π-stacked" forms.[31] The intensity of the higher energy absorbance, ascribed to the open conformation not involved in π-stacking, was found

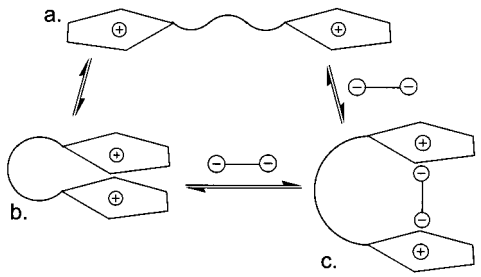


FIGURE 5. Schematic representation of solution-state conformations of sapphyrin dimer **26**: (a) open, (b) π-stacked, and (c) dicarboxylate "sandwich" complex.

to increase at the expense of the lower energy (π-stacked) band when dicarboxylate dianions were added. This observation and follow-up ^{1}H NMR analyses were considered consistent with a binding model, shown as structure (c) in Figure 5, in which the dicarboxylate dianion is "sandwiched" within the sapphyrin dimer.

Sapphyrin dimer **26** was also found to be considerably more effective than monomer **4** at enhancing the rate of transport of dicarboxylate anions through a Pressman-type U-tube membrane model. In addition, sapphyrin dimers joined by chiral linkers (e.g., **27**) were found to exhibit enantioselective recognition of N-protected amino acids, as evidenced by the association constants listed in Table 2.[31]

Further evidence for carboxylate anion recognition came in the case of sapphyrins such as **28** and **29** that contain appended carboxylic acid groups. The presence of these ionizable appendages leads to the formation of tightly held dimers, in which the core of each sapphyrin becomes protonated and associates with the anionic conjugate base "tail" of its partner.[32] The presence of these

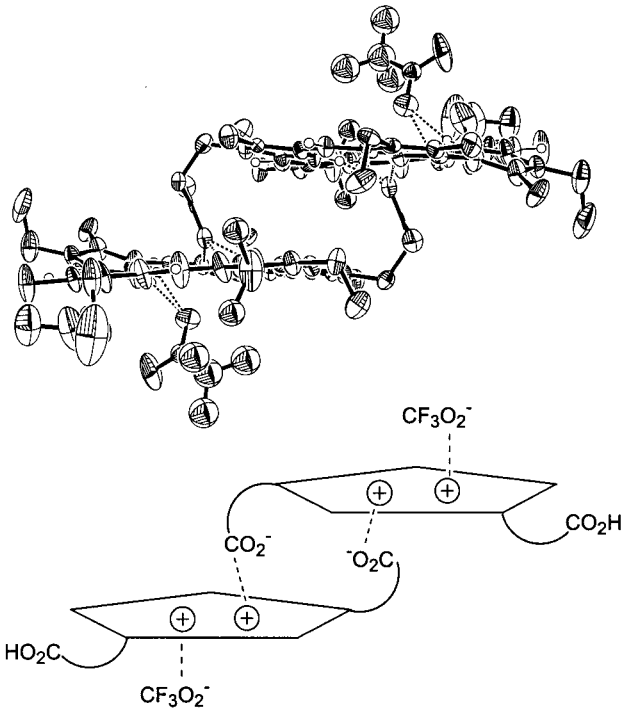


FIGURE 6. Side view of a pair of sapphyrin **28** molecules in the solid state. All hydrogen atoms (except *meso*-Hs and NHs) are removed for clarity. Hydrogen-bonding interactions are indicated with dashed lines. This figure was generated from results first published in ref 32.

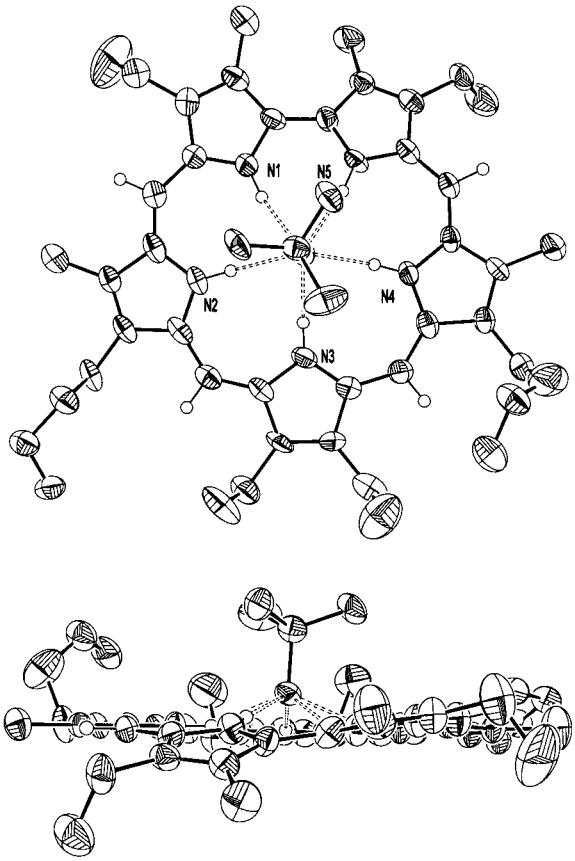


FIGURE 7. Two views of the molecular structure of the **25**·H_3PO_4 complex. All hydrogen atoms (except *meso*-Hs and NHs) are removed for clarity. Hydrogen-bonding interactions are indicated with dashed lines. This figure was generated from results first published in ref 29.

dimers in solution was confirmed by UV−vis and ^{1}H NMR spectroscopy, mass spectrometry, and vapor-phase osmometry measurements, and these dimers have been found to be the dominant species even in polar solvents (e.g., methanol) at high dilution. The proposed carboxylate anion recognition-based dimer was found to exist in the solid state as well.[32] The single-crystal X-ray structure shown in Figure 6 depicts a pair of sapphyrin **28** molecules partially overlapped with one carboxylate oxygen from each sapphyrin within hydrogen-bonding distance of the pyrrolic NHs of the other sapphyrin in the dimer. Judging from the root-mean-square planes of the nitrogen atoms, the macrocyclic planes are separated by approximately 3.39 Å. One equivalent of trifluoroacetate was also found hydrogen-bonded to each of the diprotonated sapphyrin cores.

The interactions of sapphyrins with phosphates and related anions such as phenylphosphonates and nucleotides have also been extensively studied in solution and in the solid state. The single-crystal X-ray structure of a 1:1 HPO_4^{2-}:H_2**25**$^{2+}$ complex, shown in Figure 7, typifies sapphyrin−phosphate coordination.[29] One oxygen atom from each phosphate oxyanion was found to interact via hydrogen bonds with the pyrrolic NHs of the protonated sapphyrin core. This motif has also been seen in the case of several 2:1 phosphate anion−sapphyrin complexes, in which the two anions were found to be coordinated on opposite faces of the sapphyrin macrocycle.[29]

Evidence for binding in solution includes the observation of upfield shifts in the ^{31}P NMR signals of phosphoric acid and phenyl phosphonic acid following the addition of sapphyrin.[29] Shielding effects were also observed in the ^{1}H NMR spectra of phenylphosphonic acid in the presence of sapphyrin. Using data collected from these and other spectroscopic experiments, binding affinities between sapphyrin and various phosphates were deduced. As a general rule, it was found that protonated sapphyrins bind phosphate-type anionic substrates considerably more effectively than they do chloride, bromide, and carboxylate anions, but still an order of magnitude less effectively than they do fluoride anion (Table 2).

Through use of the U-tube membrane model, it was found that protonated sapphyrin **4** acted as an effective carrier for mononucleotides under conditions where octaethylporphyrin did not.[33] The discovery that triisopropylsilyl-protected cytidine (C-Tips), added as a complementary co-transporting agent, enhanced the sapphyrin-mediated transport of GMP led to the suggestion that transport efficiency and selectivity could be improved by appending a nucleobase recognition unit onto the sapphyrin skeleton. In accord with this hypothesis, it was found that by using the cytosine- or guanosine-functionalized sapphyrins **30**−**33**, species specifically prepared for this purpose, the rate of transport for nucleotides capable of forming complementary Watson−Crick base pairs with these systems was enhanced by approximately 1 order of magnitude relative to "mismatched" nucleotides.[34,35] Pre-

sumably, the two putative recognition motifs, namely sapphyrin−phosphate binding and complementary Watson−Crick base pairing, function in concert to effect both selective and effective transport. Interestingly, selectivity was found not only among nucleotides (e.g., cytosine over guanosine or adenine in the case of a guanine-containing sapphyrin such as **32**) but also between different isomers of the same nucleotide monophosphate (e.g., 2′ over 3′ and 5′). The same trend, considered to reflect slight differences in substrate size and shape complementarity, is also manifest in the association constants of sapphyrin **31** for these phosphate anions (Table 2).[35]

The anion binding behavior of sapphyrins was further examined by functionalizing silica gel with sapphyrin **29**. The resulting support, studied in collaboration with the Iverson group, was found to allow for the HPLC-based separation of a mixture of mono-, di-, and trinucleotides under isocratic, aqueous conditions.[36] The rates of elution of AMP through a column of this functionalized silica gel in the presence of various anions (arsenate > phosphate > chloride > sulfate > nitrate = bromide > iodide > acetate) were also studied and were found to parallel the relative protonated sapphyrin−anion binding affinities observed in solution (Table 2).[37] This was considered consistent with AMP and the other anions competing for the cationic protonated sapphyrin sites present on the solid support. Inclusion of cytosine on the silica-bound sapphyrin added another dimension to the selectivity. Not only were GMP, GDP, and GTP separated from a mixture of XMP, XDP, and XTP (where X = A, C, U, and G), but the individual mononucleotides were resolved as well.[38] Thus, the feasibility of two-site recognition of anions by functionalized sapphyrins was demonstrated in the context of separations as well as transport.

The success of these experiments with simple nucleotides encouraged us, again in collaboration with Iverson group, to look at interactions between protonated sapphyrins and more complex phosphate-containing anions, such as DNA. The strong interaction between sapphyrins and DNA was demonstrated dramatically by the fact that adding sapphyrin **34** to a solution of double-stranded DNA leads to an immediate precipitation of green (and hence sapphyrin laden) fibers.[39] In analogy to what is observed in the solid-state structures of protonated sapphyrin phosphate complexes, the nature of the association in these fibers was considered to involve "phosphate chelation", a structural motif in which the sapphyrin molecules are bound to phosphate anions on the DNA backbone via multiple NH−phosphate oxyanion hydrogen bonds.[39,40] Evidence for the close association between the chiral DNA scaffold and sapphyrin was deduced from the circular dichroism (CD) spectrum of a dilute solution-phase mixture of double-stranded DNA and sapphyrin **34**, shown in Figure 8, which revealed a strong signal for the Soret-like transition of the normally achiral sapphyrin. Less intense CD signals were observed for solutions containing sapphyrin and single-stranded DNA. On the other hand, no induced CD signal was observed for

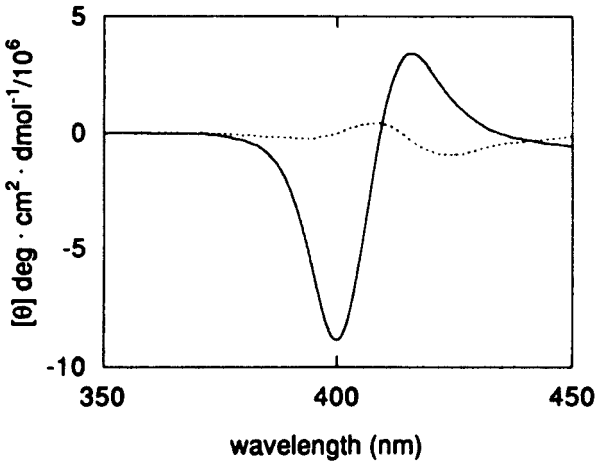


FIGURE 8. Circular dichroism (CD) spectrum of sapphyrin **34** in the presence of 10 phosphate equivalents of dsDNA (—) and ssDNA (•••) in 5 mM PIPES buffer (pH 7.0). Reproduced with permission from ref 39. Copyright 1993 Americal Chemical Society.

solutions of analogous water-soluble porphyrins with either single- or double-stranded DNA.[39]

Further evidence in support of the proposed phosphate chelation binding mode came from topoisomerase I unwinding studies.[39] In particular, under conditions where the known intercalator ethidium bromide was seen to effect the topoisomerase I-mediated unwinding of supercoiled plasmid DNA, sapphyrin **33** did not.[39] While not a proof of phosphate chelation per se, this finding helps rule out intercalation, a plausible alternative binding mode commonly seen with porphyrins.

Sapphyrin, a known singlet-oxygen-producing photosensitizer,[41] was found to catalyze the photocleavage of DNA.[42] Under conditions known to inhibit sapphyrin−DNA binding, such as high ionic strength or the added sodium dodecyl sulfate (SDS), cleavage was suppressed.[43] These findings are consistent with the proposed phosphate chelation interactions between sapphyrin and DNA and, further, support the contention that interactions between these two species play a critical role in mediating the photocleavage event.

In related work, it was found that appending an oligonucleotide conjugate to the sapphyrin allowed site-specific photocleavage of complementary strands to be achieved.[44] Such findings are important in that they support the contention that the same "two-point" binding interactions used to effect the selective transport of nucleotides (vide supra) could be used on a grander scale to effect the specific targeting of selected RNA or DNA sequences (in, e.g., so-called antisense or antigene therapy applications).

Behavior in Protic Solvents

The protonation of sapphyrin is a prerequisite for anion binding. Not surprisingly, therefore, sapphyrin has been found to be considerably more basic than porphyrin. Porphyrins generally become protonated only under relatively acidic conditions (pH < 5).[4] In contrast, the pK_a's

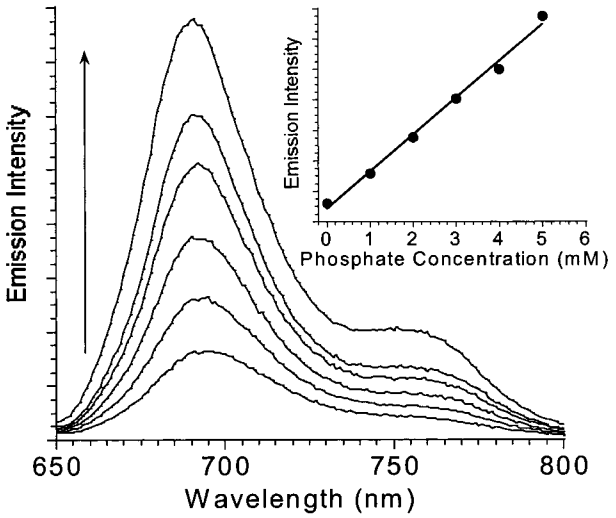


FIGURE 9. Increase in fluorescence intensity ($\lambda_{ex} = 450$ nm) when phosphate is added to a solution of sapphyrin **35** at neutral pH. Emission intensity vs phosphate concentration is shown in the inset plot.

of water-soluble sapphyrin **34** (diprotonated form) were determined to be 4.8 and 8.8 in aqueous media.[29] In a 1:1 dioxane:water mixture, the apparent pK_a's of the diprotonated form of a decaalkyl sapphyrin analogous to **4** were determined to be 5.7 and 9.8.[45] These complementary results underscore the fact that that sapphyrin exists in a cationic protonation state over a much wider and physiologically more relevant pH range than porphyrin.

Unfortunately, the chemistry of sapphyrin in polar solutions is complicated by aggregation effects. For instance, a dimerization constant of 1.2×10^4 M^{-1} has been calculated for **4**·2HCl in methanol.[41] Sapphyrins with hydrophilic appendages, such as **30**−**35**, have also been found to be extensively aggregated in water. The addition of surfactants, such as SDS, effectively breaks up these aggregates.[40] Three distinct states have been identified spectroscopically: (i) the monomer, characterized by a strong, sharp Soret-like absorbance around 450 nm; (ii) the dimer, which has a relatively broad absorbance with a lower extinction coefficient around 420 nm; and (iii) higher-order aggregates, which display a collective absorbance at ca. 410 nm.[40]

The addition of inorganic phosphate and organic phosphates to aggregated solutions of water-soluble sapphyrin **35** induces a large increase in the fluorescence emission, as shown in Figure 9. This emission enhancement is believed to be due to liberation of small amounts of the highly fluorescent monomer from the aggregates due to sapphyrin−phosphate binding interactions. The perturbation of the aggregation equilibrium is supported by the emergence of absorbances attributed to the dimeric and monomeric forms of sapphyrin (with characteristic spectral features at ca. 420 and 450 nm, respectively; vide supra).

Significant fluorescence enhancements are not observed when water-soluble sapphyrins such as **35** are treated with chloride anion. Presumably, this absence of any effect reflects the fact that the interactions between protonated sapphyrin and chloride anion are much weaker than those involving phosphate-type anions. An alternative explanation, involving chloride anion binding and heavy atom quenching, can be ruled out, as the phosphate-induced enhancements are observed even in the presence of excess chloride anion (150 mM). This lack of chloride-based competition has led us to propose that water-soluble sapphyrins might find practical utility as aggregation−deaggregation-based sensors for phosphate anions in biological milieus.[40]

Conclusions

The family resemblance between the sapphyrins and their porphyrin cousins is clear: both are aromatic, polypyrrolic macrocycles with large central polyaza cores. Since their serendipitous discovery by R. B. Woodward and coworkers, sapphyrins and heterosapphyrins with a range of different substituent patterns have been synthesized by a variety of methodologies that include rational combinations of polypyrrolic precursors as well as "one-pot" reactions. While many of the characteristics of sapphyrin are similar to those of porphyrins, sapphyrins also exhibit features that are not observed in porphyrin chemistry, such as pyrrole ring inversion, facile protonation, and anion binding.

Over the past decade the anion recognition features of the protonated forms of sapphyrin have been examined in the solid state by X-ray crystallography and in solution using techniques as diverse as optical and magnetic resonance spectroscopy, model membrane transport studies, and HPLC separation experiments. With their propensity to bind and cleave DNA, transport nucleotides, and separate various biologically important anions now established, it is conceivable that sapphyrins will have a role to play in drug development, either in the realm of purification, as adjuvants for drug delivery, or as therapeutic agents in and of themselves. Likewise, the finding that anion binding affects the spectroscopic properties and aggregation states of sapphyrins makes them attractive as potential anion sensors. In summary, therefore, we feel the interesting and potentially useful anion binding properties of sapphyrins will make them a topic of study for years to come.

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