Synthesis of a Sapphyrin–EDTA Conjugate and Preliminary Cleavage Results Using a Supercoiled Plasmid DNA Assay

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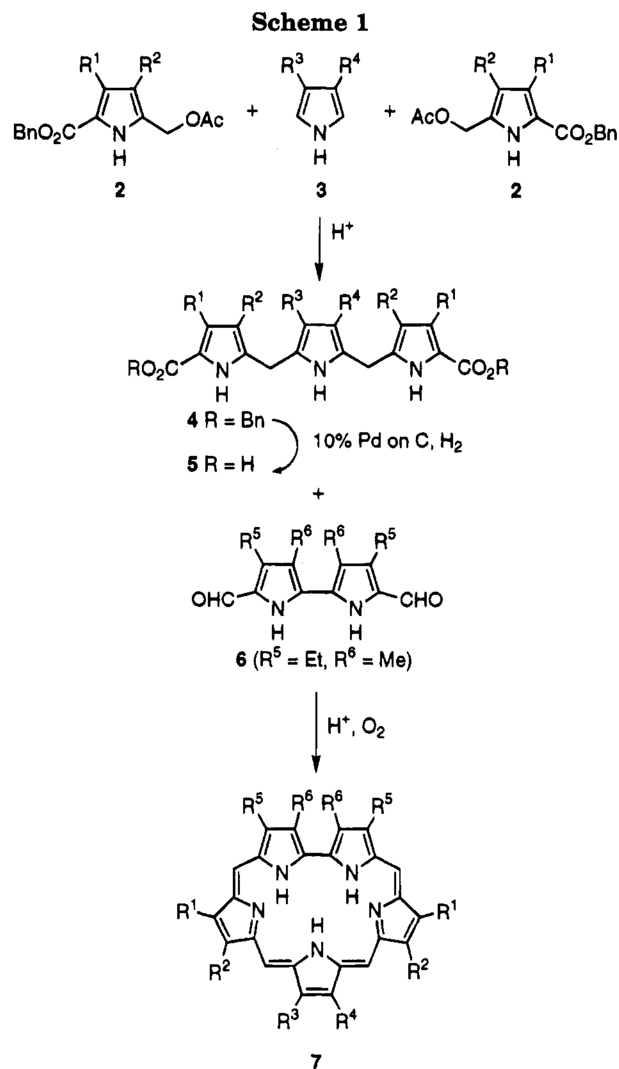
Introduction

The study of the interactions of small molecules with biological macromolecules continues to be an area of active interest. As part of our investigations into the interactions of expanded porphyrins with nucleic acids,[1] we previously reported the ability of sapphyrins, a class of pentapyrrolic, aromatic macrocycles,[2] to bind to the anionic phosphodiester backbone of DNA *via* a novel interaction we have called "phosphate chelation".[3] To investigate any potential sequence or structural selectivity of this novel binding mode, we have synthesized a sapphyrin–ethylenediaminetetraacetic (EDTA) conjugate (**1**). Previous studies of small molecule–EDTA conjugates have shown that these species, in the presence of iron(II), O_2, and a suitable reducing agent, can effect the cleavage of the DNA backbone.[4] In turn, so-called "affinity cleavage" can be used to elucidate the specificity of the small molecule–DNA interaction.[4c] In this paper we report the synthesis of **1**, as well as the results of initial DNA cleavage studies that demonstrate the augmented efficacy of this system compared to Fe·EDTA. The actual synthesis of **1** is convergent and involves two parts: the synthesis of the DNA binding agent, a sapphyrin derivative with a suitable, monofunctionalized linking unit, and the synthesis of an EDTA-like moiety for attachment to this linking group.

Results and Discussion

Sapphyrin (**7**) was originally discovered serendipitously by Woodward[5] and subsequently prepared by the research groups of Johnson[6] and Woodward[7] *via* a rational, yet somewhat tedious, synthesis. A recent synthesis of this macrocycle from our group is more straightforward[8] and has facilitated the study of this macrocycle. In general, this synthesis (see Scheme 1) involves the [2 + 3] McDonald-type condensation of a 5,5′-diformyl-2,2′-bipyrrole (e.g., **6**) with a tripyrrane diacid unit (**5**). For the synthesis of a sapphyrin–EDTA conjugate, a sapphyrin derivative that bears a peripheral functionality for attachment to an EDTA subunit was considered necessary. In principle, such a monofunctional species could be obtained from the known dicarboxylic acid derivative of sapphyrin.[9] However, to avoid undue synthetic manipulations, such as those required to monofunctionalize this doubly substituted derivative, we set out to synthesize a "mono-hook" tripyrrane (**13**) that could be elaborated in terms of preparing the monocarboxylic acid sapphryin derivative **14**. Here, the motivation for the "extra" synthetic expenditure was the realization that this same "mono-hook" tripyrrane (i.e.,

Scheme 1



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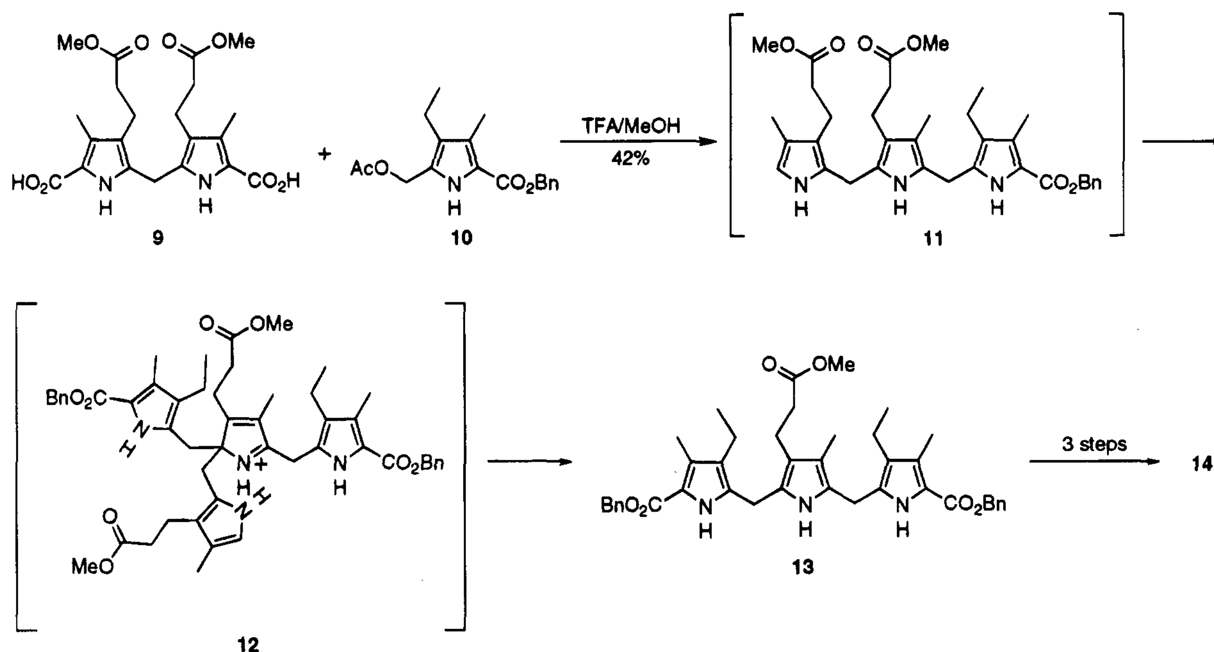
Scheme 2

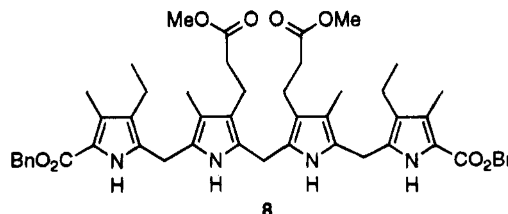


13) could find important application in the syntheses of other tripyrrane-derived expanded porphyrin macrocycles such as texaphyrin,[10] pentaphyrin,[11] and hexaphyrin.[12]

As part of our early work to develop the chemistry of expanded porphyrins, we found that tripyrranes can be obtained readily as the result of an acid-catalyzed condensation between 2 equiv of a 2-(methylacetoxy)-5-(benzyloxycarbonyl)-3,4-disubstituted-pyrrole (e.g., **2**) and 3,4-disubstituted, α-free pyrrole (e.g., **3**).[13] Tripyrranes formed by this "rational" approach can be obtained in good yield, and indeed, the desired "mono-hook" tripyrrane **13** had already been synthesized using this methodology at the time the present work was commenced.[14]

However, in an effort to design a synthetic route to the tetrapyrrolic oligomer **8**, we serendipitiously discovered an alternate route to **13** (Scheme 2). In particular, we found that heating 1 equiv of 5,5′-dicarboxy-3,3′-bis(2-(methoxycarbonyl)ethyl)-4,4′-dimethyl-2,2′-dipyrrylmethane[15] (**9**) and 2 equiv of 2-(acetoxymethyl)-5-(benzyloxycarbonyl)-4-ethyl-3-methylpyrrole[16] (**10**) at reflux in methanol under acid-catalyzed conditions gives rise to the tripyrrane **13**, rather than the expected tetrapyrrole **8**, in 42% yield.[17] In fact, this rearrangement procedure is preferred over the "rational" one of Scheme 1 for the simple reason that the starting materials are easier to prepare.

The rearrangement leading to **13** is likely to proceed *via* intermediates such as those proposed in Scheme 2. To the extent this is true, acid-catalyzed decarboxylation



of the diacid dipyrromethane **9**, followed by attack on it by 2-(acetoxymethyl)pyrrole **10** would lead to the intermediate tripyrrane **11**. Subsequent attack on **11** by another equivalent of the 2-(acetoxymethyl)pyrrole **10** then leads to the intermediate **12** which, in turn, would be expected to undergo fragmentation to yield the "mono-hook" tripyrrane **13**. In this scenario, the product distribution would be driven by the slight insolubility of **13** under the reaction conditions. Certainly, this type of rearrangement is not unheard of. In fact, similar mechanisms have been proposed before. For instance, condensation of (2-pyrrolylmethyl)pyridinium salts with lithium salts of mono-α-carboxylate pyrromethanes will give rise to hexaalkyltripyrranes.[18]

The tripyrrane **13** was used to make the corresponding "mono-hook" sapphyrin carboxylic acid **14** according the general procedure shown in Scheme 1: Intermediate **13** was debenzylated *via* hydrogenolysis using 10% Pd on carbon as the catalyst. The resulting product was then subject to decarboxylation and oxidative cyclization by treatment with 5,5′-diformyl-4,4′-diethyl-3,3′-dimethyl-2,2′-bipyrrole[8,19] (**6**) in the presence of O_2 and a catalytic amount of *p*-toluenesulfonic acid. The resulting sapphyrin methyl ester was hydrolyzed to **14** using a mixture of trifluoroacetic acid (TFA) and concentrated HCl to give an intermediate suitable for attachment to an appropriate EDTA derivative.[20] The question then became one of how to prepare the latter.

Various EDTA derivatives have been synthesized for the attachment to both small molecules and larger

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(17) When more than 2 equiv of **10** are used, yields are lowered.

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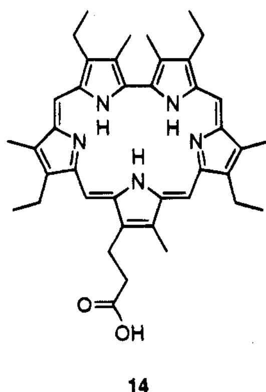
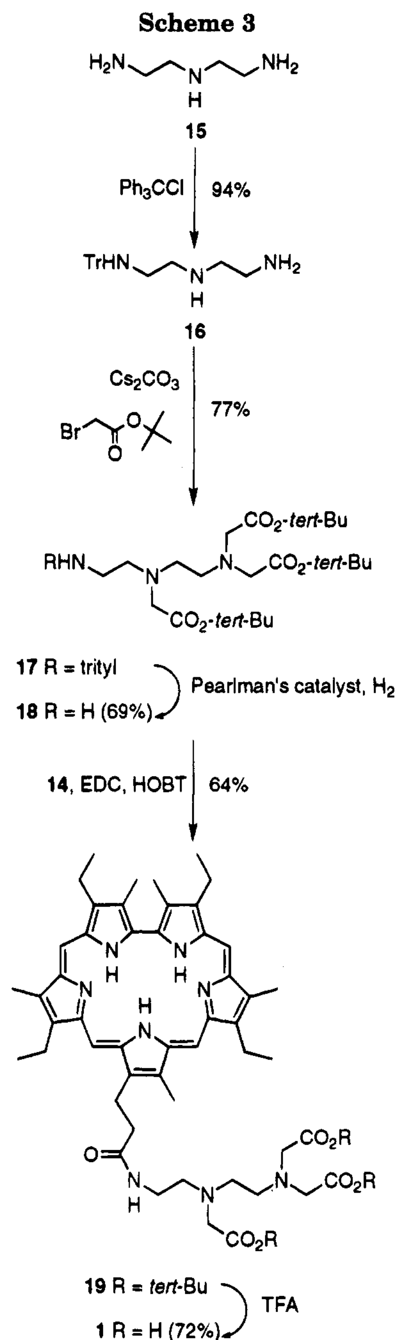


biological macromolecules to facilitate their use as biological probes.[21] These EDTA derivatives, known as "bifunctional" chelating agents, contain a functional unit for the covalent attachment of the molecule of interest as well as an EDTA moiety (often protected) for the coordinative binding of a metal species. A variety of linking functionalities have been used, including activated disulfide,[22] diazobenzene,[23] acid anhydride,[24] bromo- and iodoacetamide,[25] isothiocyanate,[25a,26] carboxylate,[27] and aniline[28] derivatives. Typically, with the exception of the latter functionality, the point of attachment is an electrophilic center and requires a nucleophile for conjugation. In some instances this is appropriate, such as when an EDTA derivative is needed for attachment to thiols or primary amines on the surface of a protein. However, in other cases, such as those where the molecule of interest contains an electrophilic functionality (e.g., the carboxylic acid in **14**), further synthetic elaboration is required. In such cases, a bis-nucleophilic reagent, such as an aliphatic diamine, has been added to one of electrophilic units to convert it into a nucleophilic species that can then, in turn, be coupled to its electrophilic target.[26c,29]

In the present instance, we chose to explore a more direct approach that utilized the bifunctional EDTA derivative **18**. The synthesis of **18** (Scheme 3) starts with commercially available diethylenetriamine (**15**). Selective N-protection of one of the primary amines on **15** was achieved in good yield (94%) by treatment with a small molar ratio of triphenylmethyl chloride (ca. 0.03 equiv). Trialkylation of this monotrityl-protected triethylenediamine with *tert*-butyl bromoacetate then yielded the triamine **17**. Selective deprotection of the trityl group was accomplished *via* hydrogenolysis in the presence of Pearlman's catalyst (20% $Pd(OH)_2$ on moist carbon). This gave the primary amine **18** in 69% yield.[30]

Scheme 3



The protected EDTA moiety **18** incorporates *tert*-butyl ester protecting groups. This is advantageous since these can be removed later under acidic conditions without damage to the sapphyrin macrocycle. In addition, **18** contains a reactive, primary aliphatic amine that can be directly conjugated to the sapphyrin acid **14**. The protected sapphyrin–EDTA conjugate **19** could thus be synthesized by coupling **14** and **18** in the presence of the standard activating agents 1-(3-(dimethylamino)propyl)-

(20) The synthesis of **14** and its methyl ester precursor has been published in preliminary form; see the Supplementary Material for Král, V.; Sessler, J. L.; Furuta, H. *J. Am. Chem. Soc.* **1992**, *114*, 8704–8705.
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(30) Attempts to produce **18** using 10% Pd on carbon or palladium black resulted in sluggish reaction times and undesired side products.

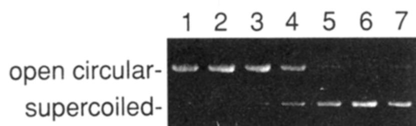

Figure 1. Photograph of a 0.8% agarose gel stained with ethidium bromide showing the results of the supercoiled pBR322 DNA cleavage assay. All reactions were run for 40 min, and the following specific reagent concentrations were employed: DNA, 15 μg/mL; DTT, 2.0 mM; PIPES, 5 mM, pH 7.0; and the specified amount of Fe·**1** or Fe·EDTA. Lane 1: 25 μM Fe·**1**. Lane 2: 13 μM Fe·**1**. Lane 3: 6.3 μM Fe·**1**. Lane 4: 3.1 μM Fe·**1**. Lane 5: 1.6 μM Fe·**1**. Lane 6: no chelate (provided as a reference). Lane 7: 25 μM Fe·EDTA.

3-ethylcarbodiimide hydrochloride and 1-hydroxybenzotriazole in DMF. Deprotection using TFA then yielded the title compound **1** in 72% yield from **19**. Because of its extremely polar nature, **1** was isolated *via* reverse phase chromatography as its organic-soluble TFA salt. The TFA salt of **1** can be converted to the water-soluble HCl salt by dissolving it in dichloromethane and treating the solution with dry HCl gas.

Once synthesized, a supercoiled plasmid DNA assay was employed to test the efficacy of **1** as a DNA binding and cleavage reagent. This assay is based on the fact that supercoiled DNA unwinds into an open circular form as the result of a single cleavage event on either strand. The resulting open circular DNA is easily separated from supercoiled (or uncleaved) DNA using electrophoresis and can be visualized by staining with ethidium bromide and subsequent illumination with UV light. Use of this assay thus allows for a comparative assessment between different cleavage reagents.[29] In this study, the iron chelate of **1** was prepared *in situ* with ferrous ammonium sulfate and found, in the presence of O_2 and the reducing agent dithiothreitol (DTT), to effect efficient cleavage of the supercoiled plasmid pBR322.

This combination of iron chelate, O_2, and reducing agent leads to so-called "Fenton" type chemistry[31] to produce what are presumed to be hydroxyl radicals that cleave the DNA−sugar backbone.[32] As can be seen in Figure 1, the DNA cleavage resulting from Fe·**1** is concentration dependent (lanes 1−5). This confirms that the observed cleavage is not the result of an impurity in the buffer or DNA sample but is a direct function of the amount of Fe·**1** present in solution. In fact, significant cleavage is seen when the conjugate concentration is as low as 3 μM (lane 4). By contrast, relatively little DNA cleavage is seen when 25 μM Fe·EDTA was employed under identical reaction conditions (compare lane 7 with lane 6).[33] These results are important because they not only serve to show that Fe·**1** is an efficacious reagent for the cleavage of DNA but also provide a proof that sapphyrin itself has a high affinity for DNA.

Currently, affinity cleavage studies are underway to determine what specificity the sapphyrin−Fe·EDTA reagent might have. Fe·**1** and its congeners by virtue of their unique phosphate recognition properties[34] could also find potential applications when it comes to investigating a range of other phosphorylated biological substrates, such as phosphorylated proteins or phospholipid-bearing entities. We are presently exploring these possibilities.

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(33) A similar comparison has been made between the iron(II) chelate complex of methidium propyl−EDTA (MPE) and Fe·EDTA (ref 29). Under aqueous, buffered conditions, no significant cleavage of pBR322 was effected by 100 μM Fe·EDTA in the presence of DTT and O_2, whereas significant cleavage was observed for Fe(II)·MPE under identical conditions in the submicromolar regime (0.1−0.01 μM).

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Experimental Section

General Procedures. Proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a QE-300 NMR spectrometer using the residual peaks in the deuterated solvents as internal standards. Elemental analysis was performed by Atlantic Microlabs. Low-resolution FAB spectra were obtained using a TSQ-70 instrument. 3-Nitrobenzyl alcohol was used as the matrix for FAB mass spectrometry. High-resolution mass spectra were recorded on a ZAB instrument. Thin layer chromatography was performed on commercially prepared silica gel plates purchased from Whatman International, Inc. Reverse phase chromatography was performed with a FPLC system (Pharmacia LKB) using a HR10/10 column packed with PepRPC 15 μm (C_2/C_{18}).

Materials. All reagents were of the highest grade available and were purchased from the Aldrich Chemical Co. unless indicated otherwise. Dichloromethane, acetonitrile, and methanol were distilled from CaH_2 under N_2. Piperazine-*N,N'*-bis-[ethanesulfonic acid] (PIPES) buffer and D,L-dithiothreitol were purchased from the Sigma Chemical Co. Agarose (electrophoresis grade) and the supercoiled plasmid pBR322 were purchased from Life Technologies (Gibco BRL). All aqueous solutions were prepared using doubly deionized water. Solutions of **1** in doubly deionized H_2O were stored at −20 °C and were thawed just prior to use.

Supercoiled Plasmid Cleavage Assay. In general, 10× stock solutions of the reagents were prepared in 5 mM PIPES, pH 7.0, and diluted to the indicated concentrations in a total reaction volume of 20 μL. The reactions were performed using the conditions outlined in Figure 1. To insure the absence of free iron, the iron chelate of the hydrochloride salt of the sapphyrin−EDTA conjugate (Fe·**1**) was prepared by mixing an equivalent of ferrous ammonium sulfate (Fisher) with 2 equiv of the ligand **1** in doubly deionized water. Fe·EDTA was prepared in an identical fashion. Upon completion of the reaction, 3 μL of a 0.25% solution of bromophenol blue in 30% glycerol/H_2O was added to each reaction and the reactions were loaded onto a 0.8% agarose gel prepared with a 2 μM solution of ethidium bromide in TEA buffer (40 mM Tris acetate, 1 mM EDTA). After electrophoresis, the DNA was visualized using a 312 nm UV lightbox.

2,5-Bis((5-(benzyloxycarbonyl)-3-ethyl-4-methylpyrrol-2-yl)methyl)-3-((methoxycarbonyl)ethyl)-4-methylpyrrole (13). A solution of 5,5'-dicarboxy-3,3'-bis((2-methoxycarbonyl)ethyl)-4,4'-dimethyl-2,2'-dipyrrylmethane[15] (**9**, 5.0 g, 12 mmol), 2-(acetoxymethyl)-5-(benzyloxycarbonyl)-4-ethyl-3-methylpyrrole[16] (**10**, 7.3 g, 23 mmol), *p*-toluenesulfonic acid monohydrate (0.46 g, 2.4 mmol), and trifluoroacetic acid (9.3 mL, 0.12 mmol) in MeOH was heated at reflux in the dark for 2 h, whereupon the solution turned a dark brown color. The solution was cooled to room temperature, and half of the solvent was removed using rotary evaporation. The solution was allowed to stand overnight at −10 °C. Black solids precipitated out overnight and were collected. Recrystallization from MeOH yielded 3.3 g (42%) of an air- and light-sensitive, reddish-brown solid: ^{1}H NMR δ ($CDCl_3$) 0.94 (3H, t), 0.99 (3H, t), 2.04 (3H, s), 2.21 (3H, s), 2.27 (3H, s), 2.29−2.48 (6H, m), 2.79 (2H, t), 3.41 (2H, s), 3.62 (2H, s), 3.66 (3H, s), 4.36 (2H, s), 4.41 (2H, s), 6.97−6.99 (5H, m), 7.23−7.25 (5H, m), 8.76 (1H, b), 10.93 (1H, b), 11.18 (1H, b); ^{13}C NMR δ ($CDCl_3$) 9.3, 10.9, 11.0, 15.6, 15.8, 17.1, 17.2, 20.2, 21.9, 22.0, 35.7, 60.2, 65.2, 111.6, 115.5, 117.0, 117.4, 123.0, 123.2, 123.2, 123.4, 126.5, 127.2, 128.1, 132.4, 133.1, 136.9, 162.6, 162.7, 173.6; MS FAB (M + 1)$^+$ 678 *m/z*; HRMS, M$^+$ 677.3460 (calcd for $C_{41}H_{47}N_3O_6$ 677.3465).

***N*-(2-((Triphenylmethyl)amino)ethyl)-1,2-ethanediamine (16).** To a solution of diethylenetriamine (**15**, 22.0 mL, 210 mmol) in 200 mL of dry, argon-purged dichloromethane at 0 °C was added triphenylmethyl choride (1.8 g, 6.5 mmol). A white precipitate formed shortly after the addition, and the solution was allowed to stir at 0 °C overnight. The organic layer was washed with 1 M NaOH (3 × 200 mL) and dried over Na_2SO_4 before the solvent was removed by means of a rotary evaporator. Purification on silica gel, neutralized with a drop of triethylamine, using a gradient of CH_3OH/CH_2Cl_2 as the eluent yielded 2.1 g (94%) of a viscous, clear oil: ^1H NMR δ (CD_2Cl_2) 2.27 (2H, t), 2.55 (2H, t), 2.70 (4H, m), 7.23–7.65 (15H, m); ^{13}C NMR δ ($CDCl_3$) 41.5, 43.1, 49.9, 52.1, 70.7, 126.1, 127.7, 128.4, 146.1; MS FAB $(M + H)^+$ m/z 346; HRMS $(M + H)^+$ 346.2278 (calcd for $C_{23}H_{28}N_3$ 346.2283).

***N,N,N'*-Tris((*tert*-butoxycarbonyl)methyl)-*N'*-(2-((triphenylmethyl)amino)ethyl)-1,2-ethanediamine (17).** *tert*-Butyl bromoacetate (4.2 mL, 26 mmol) was added dropwise to a suspension of *N*-(2-((triphenylmethyl)amino)ethyl)-1,2-ethanediamine (**16**, 3.0 g, 8.7 mmol) and Cs_2CO_3 (8.45 g, 26 mmol) in 40 mL of dry acetonitrile at 4 °C under argon. The resulting mixture was stirred vigorously at 4 °C for 12 h. The inorganic salts were removed by vacuum filtration, and the solvent was removed by means of a rotary evaporator. Purification on silica gel, neutralized with a drop of triethylamine, using a gradient of CH_2Cl_2/hexanes as the eluent yielded 4.6 g (77%) of a clear, viscous oil: ^1H NMR δ (CD_2Cl_2) 1.41 (9H,s), 1.44 (18H, s), 2.21 (2H, t), 2.62 (2H, t), 2.72 (4H, m), 3.11 (2H, s), 3.36 (4H, s), 7.19–7.51 (15H, m); ^{13}C NMR δ ($CDCl_3$) 27.9, 28.1, 41.1, 52.6, 52.8, 54.5, 55.6, 56.1, 70.6, 80.8, 126.1, 127.7, 128.7, 146.3, 170.6, 170.9; MS FAB $(M + H)^+$ 688; HRMS $(M + H)^+$ 688.4323 (calcd for $C_{41}H_{58}N_3O_6$ 688.4326).

***N,N,N'*-Tris((*tert*-butoxycarbonyl)methyl)-*N'*-(2-aminoethyl)-1,2-ethanediamine (18).** A suspension of *N,N,N'*-tris((*tert*-butoxycarbonyl)methyl)-*N'*-(((2-triphenylmethyl)amino)ethyl)-1,2-ethanediamine (**17**, 380 mg, 0.57 mmol) and Pearlman's catalyst (20% $Pd(OH)_2$ on moist carbon, 1.0 g) was stirred in 15 mL of 30% ethyl acetate/methanol for 5 h under an atmosphere of hydrogen gas. The catalyst was removed by vacuum filtration (CAUTION: Pearlman's catalyst is pyrophoric!), and the solvent was removed by means of a rotary evaporator. Purification on silica gel, neutralized with a drop of triethylamine, using a gradient of CH_3OH/CH_2Cl_2 as the eluent yielded 176 mg (69%) of a light tan solid: ^1H NMR δ ($CDCl_3$) 1.42 (27H, s), 2.14 (2H, b), 2.69 (4H, m), 2.77 (4H, m), 3.27 (2H, s), 3.42 (4H, s); ^{13}C NMR δ ($CDCl_3$) 28.1, 39.9, 52.3, 52.8, 56.1, 56.2, 57.1, 80.8, 80.9, 170.6, 171.1; MS FAB $(M + H)^+$ m/z 446; HRMS $(M + H)^+$ 446.3242 (calcd for $C_{22}H_{44}N_3O_6$ 446.3230).

3,8,17,22-Tetraethyl-12-[2-((2-(*N,N,N'*-tris((*tert*-butoxycarbonyl)methyl)-1,2-ethanediamino)ethyl)amino)carbonyl)ethyl]-2,7,13,18,23-pentamethylsapphyrin (19). A solution of the dihydrochloride salt of the "mono-hook" sapphyrin carboxylic acid[20] (**14**, 35 mg, 0.050 mmol), *N,N,N'*-tris((*tert*-butoxycarbonyl)methyl)-*N'*-(2-aminoethyl)-1,2-ethanediamine (**18**, 25 mg, 0.056 mmol), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (12 mg, 0.063 mmol), and 1-hydroxybenzotriazole hydrate (9 mg, 0.067 mmol) in 1.0 mL of dry *N,N*-dimethylformamide was allowed to stand for 10 h. Then 50 mL of CH_2Cl_2 was added and the organic layer washed with 50 mL of brine, 50 mL of H_2O, and again with 50 mL of brine. The organic layer was dried over Na_2SO_4 before the solvent was removed under high vacuum. Purification on silica gel using a gradient of $MeOH/CH_2Cl_2$ as the eluent then afforded 36 mg (64%) of a blue solid. The macrocycle can be further purified by recrystallization from CH_2Cl_2/pentane: UV/vis ($CHCl_3$) [λ_{max}, nm]: 434.5 (s), 456.0, 575.0, 623.0, 676.0 (s); ^1H NMR δ ($CDCl_3$) −4.95 (1H, s), −4.90 (1H, s), −4.51 (1H, s), −4.27 (2H, s), 1.35 (18H, s), 1.41 (9H, s), 2.26 (12H, m), 3.32 (2H, s), 3.54 (4H, s), 3.61 (4H, m), 4.15 (6H, s), 4.26 (6H, s), 4.31 (3H, s), 4.58 (4H, q), 4.74 (4H, m), 5.13 (2H, t), 7.60 (1H, b), 11.66 (1H, s), 11.73 (2H, s), 11.83 (1H, s); ^{13}C NMR δ ($CDCl_3$) 12.5, 12.9, 15.7, 17.7, 17.8, 20.8, 23.3, 27.9, 27.9, 37.8, 39.1, 51.6, 51.8, 52.3, 55.8, 55.9, 80.8, 80.9, 91.6, 91.9, 98.0, 126.7, 129.2, 129.3, 129.9, 132.5, 132.6, 134.9, 135.1, 135.5, 136.4, 137.2, 140.6, 141.2, 141.5, 141.9, 170.4, 171.2, 171.9; MS FAB, M^+ 1057; HRMS, M^+ 1056.6790 (calcd for $C_{62}H_{88}N_8O_7$ 1056.6776).

3,8,17,22-Tetraethyl-12-[2-(((2-(*N,N,N'*-tris(carboxymethyl)-1,2-ethanediamino)ethyl)amino)carbonyl)ethyl]-2,7,-13,18,23-pentamethylsapphyrin (1). Compound **19** was dissolved in 15 mL of trifluoroacetic acid and was allowed to stand at room temperature in the dark for 4 h. The solvent was removed using a rotary evaporator and the product recrystallized from CH_2Cl_2/pentane. Further purification *via* reverse phase chromatography was performed using a 40 min gradient from 75% 0.07% TFA/CH_3CN:doubly deionized H_2O to 100% 0.07% TFA/CH_3CN. The product eluted as a single bright green band toward the end of the gradient and, when dried under high vacuum, yielded 28 mg (72%) of a blue solid: MS FAB $(M + H)^+$ m/z 890; HRMS $(M + H)^+$ 889.4962 (calcd for $C_{50}H_{65}N_8O_7$ 889.4976). Anal. Calcd for $C_{50}H_{64}N_8O_7{\cdot}2TFA{\cdot}2H_2O$: C, 56.24; H, 6.12; N, 9.72. Found: C, 56.22; H, 5.87; N, 9.49. Upon the addition of ferrous ammonium sulfate, mass spectrometric results were obtained that were consistent with the formation of Fe·1: MS FAB m/z 943; HRMS 943.4162 (calcd for $C_{50}H_{63}N_8O_7{}^{56}Fe$ 943.4169).

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