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Ex Post Glycoconjugation of Phthalocyanines

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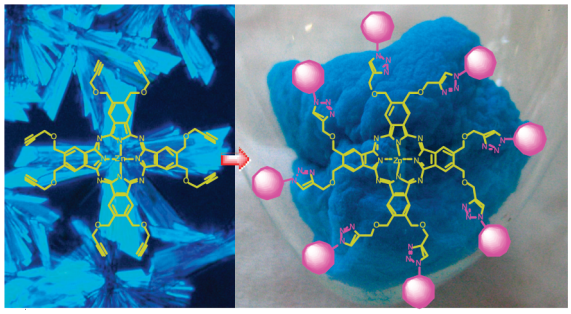
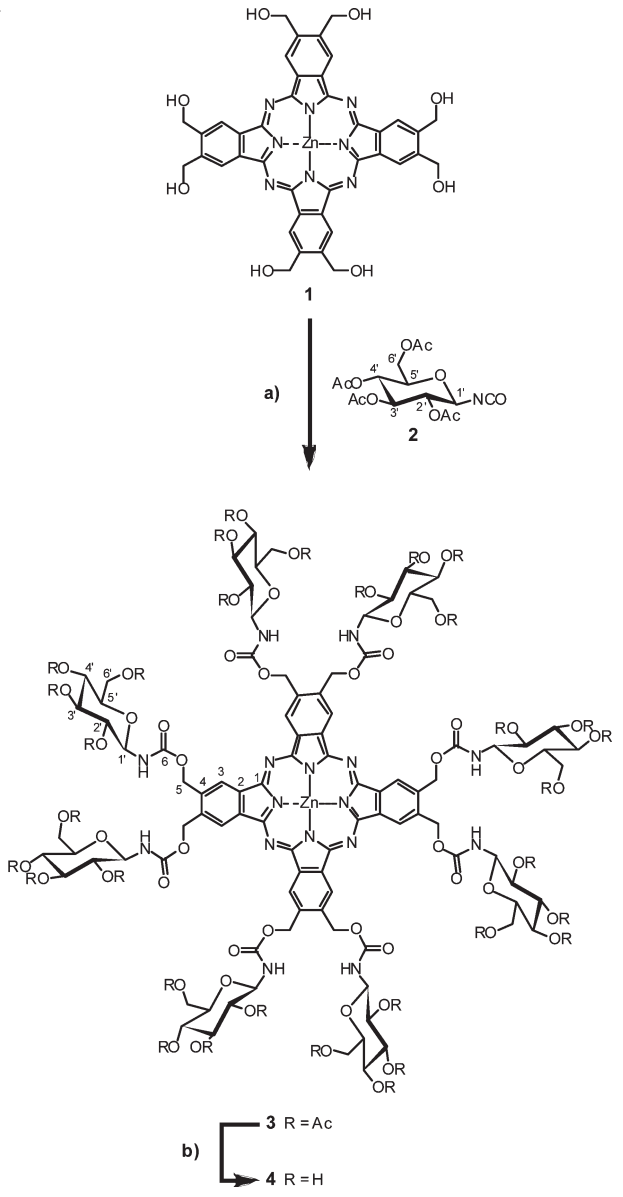


For the first time, fully fledged phthalocyanines (Pc*s*) were ex post glycoconjugated, that is, via 1,3-dipolar cycloaddition reaction. This divergent approach gains rapid access to a broad range of highly diverse Pc*s* bearing chemically sensitive substituents. This will be a breakthrough in generating structure−activity relationships (SAR) for the development of novel bioactive molecules.

Phthalocyanines (Pc*s*) are extremely interesting structures for a plethora of applications in material and life sciences, such as for applications in nonlinear optics (including optical limitation), xerography (as photoconductors), optical data storage (as the laser absorption layer within recordable compact discs)[1] molecular electronics, solar energy conversion, catalysis, gas sensors, and as diagnostic imaging agents[2] as well as emerging therapeutics in photodynamic cancer therapy[3] (PDT).[4]

For some time past, glycosylated or glycoconjugated Pc*s* attracted attention as potential cell specific agents.[5] The Pc core was conceptualized as a scaffold for spatially aligning carbohydrate motifs in a quasi antennary arrangement to achieve polyvalent or cooperative binding effects[6] for mimicking lectin interactions.[7] The synthetic access to these Pc*s*

SCHEME 1. Carbamoylation of ZnPc 1 and Deprotection[a]



[a]Reagents and conditions: (a) 2 equiv glucopyranosyl isocyanate **2**, py, 50 °C, 64 h, 82%; (b) NaOMe in MeOH (∼pH 9), DMF, rt, 72 h, 99%.

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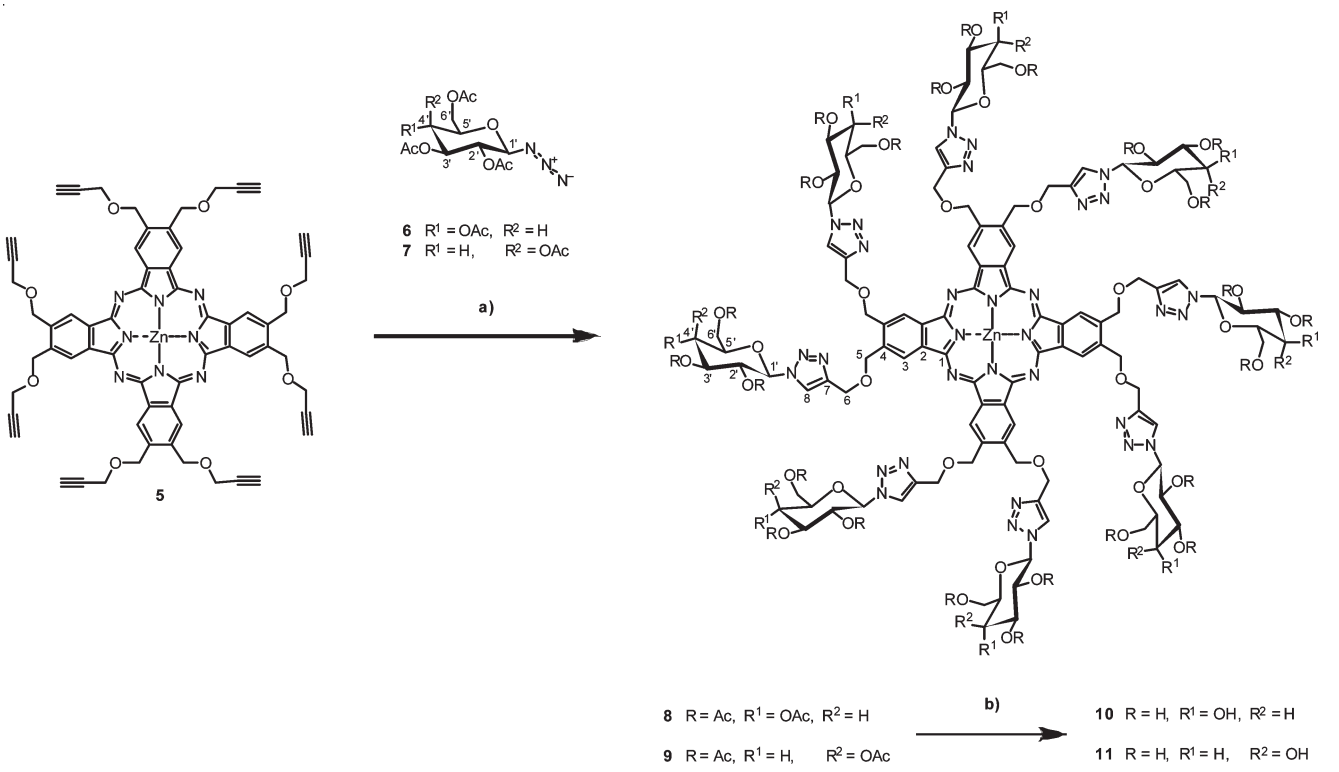
SCHEME 2. 1,3-Dipolar Cycloaddition Reaction ("Click chemistry") of Glucopyranosyl Azide 6 and Galactopyranosyl Azide 7 with ZnPc-*op*-$CH_2OCH_2C{\equiv}CH$ (5)[11a]



[a]Reagents and conditions: (a) ZnPc **5**, THF/MeOH = 2:1, 0.16 equiv $CuSO_4 \cdot H_2O$, 0.5 equiv (+) sodium L-ascorbate, 3 equiv glucopyranosyl azide **6**, rt, light shielded, 18 h, 81%; ZnPc **5**, THF/MeOH = 2:1, 0.3 equiv $CuSO_4 \cdot H_2O$, 0.8 equiv (+) sodium L-ascorbate, 5 equiv galactopyranosyl azide **7**, rt, light shielded, 18 h, 72%.[12] (b) NaOMe in MeOH (~pH 11), DMF, rt, 6 h, 89−99%.

was effected via cyclotetramerization of an appropriately substituted phthalic acid or phthalodinitrile derivative. Albeit much success has been achieved over time in attenuating the harsh cyclization conditions, the high temperatures and strong bases in use are mostly detrimental to chemically delicate substituents. Consequently, examples of complexly decorated Pc*s* are scarce and structural diversity is severely restricted.

Obviously, these convergent approaches are also unfavorable for facile modifications as needed for effectively generating structure/activity relationships (SAR) because for each structural alteration the synthetic sequence has to start over from scratch. Curiously, little effort has been made to overcome these obstacles by employing appropriate protective group chemistry and, after deprotection, to diversify the resulting fully fledged Pc scaffold by ex post application of suitable linker chemistry.[8]

Recently we reported a fully protected Pc, which was conveniently deprotected and subsequently derivatized. The resulting Pc scaffold **1** and, in particular, the successive 8-fold alkynylated Pc platform **5** are ideally suited for ex post decoration of a fully fledged Pc with delicate substituents.[9]

To prove the viability of this concept, we have chosen the ex post glycoconjugation of the above Pc scaffold. As a first example, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isocyanate (**2**)[10] was cleanly reacted with **1** under very mild conditions, yielding the *octa*-substituted glucoconjugated ZnPc **3** as a single isomer under complete retention of stereochemistry (Scheme 1). X-ray analyses of the isocyanate **2** and the precursor 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl amine (**2b**) confirmed the β-configuration (see Figures S35 and S36 in the Supporting Information). Deprotection of the *O*-acetyl-protected carbamoyl glucosyl ZnPc **3** was successfully achieved under Zemplén conditions. Acetyl groups were completely removed, whereas all carbamate linkages remained intact, thus, isolating **4** as a pure uniform substance as proven by ^1H and ^{13}C NMR and HRMS (see figures in the Supporting Information). Furthermore, the coupling constant, namely 8.4 to 9.4 Hz, at the anomeric proton H-1′ of ZnPc*s* **3** and **4** were in agreement with complete retention of the β-configuration (see Experimental Section and Table S1 in the Supporting Information).

However, from a medicinal chemistry point of view, it might be argued that carbamate linkages, aside from prodrug approaches, are unsuitable due to their putative metabolic liabilities and therefore should be replaced by metabolically more stable linkages, that is, by ethers.

Intentionally, with the *octa*-propargyloxy derivative **5** in hand,[9] access to a plethora of cycloaddition reactions is gained. In particular, the 1,3-dipolar cycloaddition of azides with alkynes, originally introduced by Huisgen[13a] has proven itself as highly versatile for the functionalization of biologically

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interesting molecules.[13b] By the addition of Cu^{I}, regioselectivity was independently improved by Meldal[13c] and Sharpless[13d] and is nowadays well recognized as "Click-Chemistry". Consequently, the decoration of **5** with galactose and glucose by "clicking" the corresponding azido pyranoses was attempted (Scheme 2).

The rationale behind choosing Glc and Gal was the well documented affinity of antennary β-Gal structures to the hepatic asialoglycoprotein receptor (ASGP-R). In contrast, the glucosylated analog should not be recognized by this receptor and therefore might serve as a negative control.[14]

The readily synthesized Glc-azide **6** and Gal-azide **7** again were isolated as pure β-anomers, as confirmed by ^{1}H NMR and X-ray analyses (see Figures S34 and S37 in the Supporting Information).

To our delight, regiospecific copper(I)-catalyzed cycloaddition exclusively transformed **5** into the 8-fold 1,4-substituted triazole isomers **8** and **9**. Simple flash chromatography provided the isomerically pure 8-fold glycopyranosyl conjugated ZnPc*s* in high yields. NMR, MALDI-TOF, and ESI-HRMS analyses were in full agreement with the expected structures (see the Supporting Information). NOESY-NMR and coupling constants unambiguously confirmed β-configurations at the anomeric centers (H-1′) of the pyranose units. ^{1}H NMR of the peracetylated derivative **9** is exemplarily shown in Figure 1a. Deprotection under Zemplén conditions proceeded smoothly, thus, quantitatively yielding the final target structures **10** and **11** (Figure 1b) under retention of β-configurations at the anomeric centers (see Table S2 in the Supporting Information). The remarkably well-resolved NMR peaks in combination with the sharp Q-band and high Q-/B-band ratio[15] in the UV−vis spectra indicated that the protected, as well as the unprotected, ZnPc*s* **3**, **4**, **8**, **9**, **10**, and **11** exhibited only marginal aggregation behavior in DMSO solutions[5a,c,16] (see Figures S29 and S30 in the Supporting Information).

As expected, measurements of optical activity were futile due to the high extinction coefficients of Pc compounds at 589 nm. Instead, circular dichroism (CD) spectra of 12−13 μM solutions of Pc compounds in DMSO or THF were recorded at wavelengths between 800 and 300 nm. Interestingly, the glucosyl conjugated ZnPc*s* **3** and **8** showed distinct circular dichroism.[17] In contrast, to our surprise, the CD effect of the galactosyl conjugated ZnPc **9** was marginal. The CD spectra of achiral ZnPc **1** and alkynylated ZnPc **5** were recorded as negative controls (see Supporting Information, Figures S31−S33).

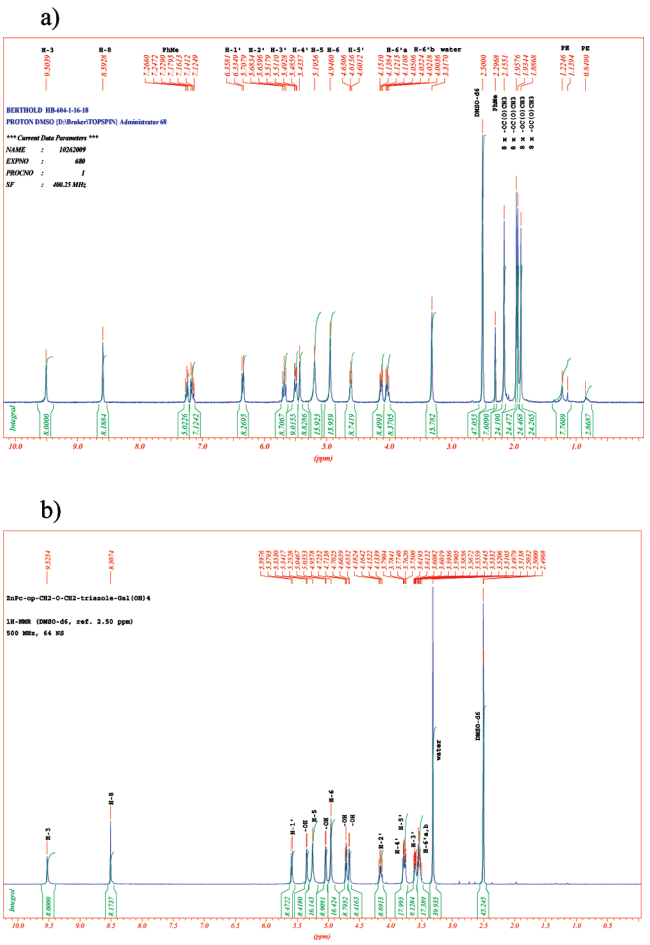

FIGURE 1. ^{1}H NMR spectra of (a) peracetylated 8-fold galactopyranosyl conjugated ZnPc **9**. Toluene (PhMe) peaks origin from workup procedure; (b) deprotected 8-fold galactopyranosyl conjugated ZnPc **11**.

The biological effects of the novel *octa*-glycosylated Pc*s* are under investigation and will be reported elsewhere.

In summary, for the first time ex post substitution of a full-fledged ZnPc scaffold **1** and ZnPc **5** with carbohydrates has been demonstrated. The linker chemistries applied herewith are of general scope and optimally suited to be extended to a plethora of sensitive bioactive structures,[18] such as amino acids, peptides,[19] proteins,[20] nucleic acids,[21] and steroids.[22] This ex post derivatization of ZnPc*s* not only will pave the way to the rapid and effective synthesis of highly diverse Pc*s* for biological testing but also toward the integration of Pc cores into functional supramolecular biological matrices, as nature did successfully for porphyrins from the origin of life.

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Experimental Section

Synthesis of {2,3,9,10,16,17,23,24-Octakis[*O*-(*N*-1-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)carbamoyloxy)methyl]phthalocyaninato} Zinc(II) (3). ZnPc-*op*-CH_2OH (**1**; 51 mg, 60 μmol) was dissolved in dry pyridine (5.0 mL, 62 mmol) in a screw cap vial by means of sonification and 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl isocyanate (**2**; 0.38 g, 1.0 mmol) was quickly added. The mixture was stirred in a preheated aluminum block at 50 °C for 64 h. The solvent was evaporated and the remaining residue (0.46 g) was purified by column chromatography on silica gel (gradient from EA → 10% MeCN). Evaporation of the solvent yielded the peracetylated glucosyl carbamate ZnPc **3** as blue, shiny plates (190 mg, 50 μmol, 82%). Mp 276 °C; R_f 0.22 (EA); $[\alpha]^{20\ °C}_{589nm}$ transmission too low; UV−vis (DMSO) λ_{max} (log ε) = 680 (5.39), 613 (4.61), 351 (4.86); ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C) δ 9.55 (br s, 8H, H-3), 8.64 (br d, $^3J_{H\text{-}1',NH}$ = 8.9 Hz, 8H, NH), 5.83 (d, $^2J_{H\text{-}5a,H\text{-}5b}$ = 12.7 Hz, 8H, H-5a), 5.77 (d, $^2J_{H\text{-}5a,H\text{-}5b}$ = 14.5 Hz, 8H, H-5b), 5.39 (dd, $^3J_{H\text{-}2',H\text{-}3'}$ = 9.4 Hz, $^3J_{H\text{-}3',H\text{-}4'}$ = 9.7 Hz, 8H, H-3′), 5.32 (dd, $^3J_{H\text{-}1',NH}$ = $^3J_{H\text{-}1',H\text{-}2'}$ = 9.4 Hz, 8H, H-1′), 4.97 (dd, $^3J_{H\text{-}1',H\text{-}2'}$ = 9.2 Hz, $^3J_{H\text{-}2',H\text{-}3'}$ = 9.4 Hz, 8H, H-2′), 4.90 (dd, $^3J_{H\text{-}3',H\text{-}4'}$ = $^3J_{H\text{-}4',H\text{-}5'}$ = 9.7 Hz, 8H, H-4′), 4.19−4.13 (m, 16H, H-5′/H-6′a), 3.99 (d, $^2J_{H\text{-}6'a,H\text{-}6'b}$ = 10.7 Hz, 8H, H-6′b), 1.98, 1.96, 1.95, 1.92 (s, 4 × 24H, 4 × -OC(O)CH_3); ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C) δ 169.9, 169.5, 169.2, 169.0 (4 × -O$\underline{C}$(O)CH_3), 155.6 (C-6), 153.1 (C-1), 137.7 (C-2), 137.1 (C-4), 123.6 (C-3), 79.8 (C-1′), 72.9 (C-3′), 71.9 (C-5′), 70.5 (C-2′), 67.8 (C-4′), 64.3 (C-5), 61.7 (C-6′), 20.4, 20.3, 20.24, 20.23 (4 × -OC(O)$\underline{C}H_3$); MALDI-TOF (anthracene-1,8,9-triol, *m*/*z* (%)) calcd for $C_{160}H_{185}N_{16}O_{88}Zn^+$, 3805.63; found, 3807.00 (100), 3807.98 (96), 3806.09 (95), 3808.96 (84), 3805.15 (69), 3809.87 (61) $[M + H]^+$; HRMS (ESI) *m*/*z* calcd for $C_{160}H_{186}N_{16}O_{88}Zn^{2+}$, 1901.493; found, 1901.492 $[M + 2 \times H]^{2+}$; Anal. Calcd for $C_{160}H_{184}N_{16}O_{88}Zn \cdot 4H_2O$: C, 49.57; H, 4.99; N, 5.78. Found: C, 49.66; H, 5.08; N, 5.67.

Synthesis of {2,3,9,10,16,17,23,24-Octakis[((*N*-1-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-methyl]phthalocyaninato} Zinc(II) (9). To a vigorously stirred solution of peralkynylated ZnPc **5** (33 mg, 29 μmol) in THF (8 mL) and MeOH (4 mL) was added consecutively $CuSO_4 \cdot 5H_2O$ (17 mg, 68 μmol), (+)sodium L-ascorbate (36 mg, 180 μmol), and 2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl azide (**7**; 0.457 mg, 1.22 mmol). After 18 h of stirring in the dark at rt, all solvents were removed and the residue (611 mg) was purified by column chromatography on silica gel (THF/PE, 60:40 → THF). The combined fractions containing **9** were evaporated and the residue (93 mg) was dissolved in a minimum of THF (3 mL) and precipitated in MeOH/water to yield **9** after centrifugation and evaporation with PhMe (86 mg, 21 μmol, 72%). Mp 155−167 °C (240 °C dec.); R_f 0.90 (25:75, PE/acetone), 0.83 (30:70, PE/acetone); $[\alpha]^{20\ °C}_{589nm}$ transmission too low; UV−vis (DMSO) λ_{max} (log ε) = 680 (5.44), 613 (4.67), 354 (4.95); ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C) δ 9.50 (br s, 8H, H-3), 8.59 (br s, 8H, H-8), 6.34 (d, $^3J_{H\text{-}1',H\text{-}2'}$ = 9.3 Hz, 8H, H-1′), 5.68 (dd, $^3J_{H\text{-}1',H\text{-}2'}$ = 9.5 Hz, $^3J_{H\text{-}2',H\text{-}3'}$ = 9.8 Hz, 8H, H-2′), 5.50 (dd, $^3J_{H\text{-}2',H\text{-}3'}$ = 10.0 Hz, $^3J_{H\text{-}3',H\text{-}4'}$ = 2.8 Hz, 8H, H-3′), 5.44 (br s, 8H, H-4′), 5.20 (br s, 16H, H-5), 4.95 (br s, 16H, H-6), 4.62 (br dd, $^3J_{H\text{-}5',H\text{-}6'a}$ = $^3J_{H\text{-}5',H\text{-}6'b}$ = 5.8−6.0 Hz, 8H, H-5′), 4.13 (dd, $^2J_{H\text{-}6'a,H\text{-}6'b}$ = 11.0 Hz, $^3J_{H\text{-}5',H\text{-}6'a}$ = 5.0−6.8 Hz, 8H, H-6′a), 4.03 (dd, $^2J_{H\text{-}6'a,H\text{-}6'b}$ = 11.6 Hz, $^3J_{H\text{-}5',H\text{-}6'b}$ = 7.3 Hz, 8H, H-6′b), 2.16, 1.96, 1.93, 1.89 (s, 4 × 24H, 4 × -OC(O)$\underline{C}H_3$); ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C) δ 169.9, 169.8, 169.4, 168.6 (4 × -O$\underline{C}$(O)CH_3), 153.2 (C-1), 144.6 (C-7), 138.7 (C-4), 137.4 (C-2), 123.6 (C-8), 122.6 (C-3), 84.4 (C-1′), 72.9 (C-5′), 70.4 (C-3′), 69.6 (C-5), 67.9 (C-2′), 67.3 (C-4′), 63.1 (C-6), 61.5 (C-6′), 20.4, 20.3, 20.2, 20.0 (4 × -OC(O)$\underline{C}H_3$); MALDI-TOF (anthracene-1,8,9-triol) calcd for $C_{176}H_{201}N_{32}O_{80}Zn^+$, 4110.04; $C_{176}H_{200}N_{32}NaO_{80}Zn^+$, 4132.02; $C_{176}H_{200}KN_{32}O_{80}Zn^+$, 4148.13; found *m*/*z*, 4109.1 $[M + H]^+$, 4132.2 $[M + Na]^+$, 4149.5 $[M + K]^+$. HRMS (ESI^+) *m*/*z* calcd for $C_{176}H_{202}N_{32}O_{80}Zn^{2+}$, 2053.600; found, 2053.603 $[M + 2 \times H]^{2+}$; Anal. Calcd for $C_{176}H_{200}N_{32}O_{80}Zn \cdot 6H_2O \cdot 2PhMe$: C, 51.85; H, 5.22; N, 10.18. Found: C, 51.83; H, 5.20; N, 10.33.

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Supporting Information Available: General experimental methods, additional experimental procedures, compound characterization data, copies of spectra (NMR, MALDI-TOF, HRMS-ESI, UV−vis, CD), crystallographic information files (CIFs), thermal ellipsoid plots, and additional tables. This material is available free of charge via the Internet at http://pubs.acs.org.