Syntheses and Energy Transfer in Multiporphyrin Arrays Self-Assembled with Hydrogen Bonding Recognition Groups and Comparison with Covalent Steroidal Models

Teodor Silviu Balaban,[a,b]* Nina Berova,[c]* Charles Michael Drain, [d,e]* Robert Hauschild, [b,f] Xuefei Huang, [c] Heinz Kalt, [b,f]* Sergei Lebedkin, [a] Jean-Marie Lehn, [a,g] Fotis Nifaitis, [d] Gennaro Pescitelli, [c,h] Valentyn I. Prokhorenko, [i] Gernot Riedel, [f] Gabriela Smeureanu, [d] and Joachim Zeller[b,f]

[a] Karlsruhe Institute of Technology (KIT), Forschungszentrum Karlsruhe (FZK), Institute for Nanotechnology (INT), Postfach 3640, D-76021 Karlsruhe, Germany, FAX: (internat.) + 49 724 782 8298; e-mail: silviu.balaban@int.fzk.de

[b] Center for Functional Nanostructures (CFN), University of Karlsruhe, Wolfgang-Gaede-Str.1, D-76131 Karlsruhe, Germany

[c] Department of Chemistry, Columbia University, Havermeyer Hall, MC 3114, 3000 Broadway, New York, NY 10027, USA. FAX (internat) + 1 212 932 8273, e-mail: ndb1@columbia.edu

[d] Department of Chemistry and Biochemistry, Laboratory of Supramolecular Photonics, Hunter College and Graduate Center of the City University of New York, 695 Park Ave, New York, NY 100021. FAX. (internat) +1 212 772 5332, e-mail: cdrain@hunter.cuny.ed

[e]The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA

[f] Institute of Applied Physics, University of Karlsruhe, Wolfgang-Gaede-Str.1, D-76131, Karlsruhe, Germany, FAX: (internat.) +49 721 608 8480, e-mail: heinz.kalt@physik.unikarlsruhe.de

[g] ISIS, Université Louis Pasteur, 8 allée Gaspard Monge, F-67000 Strasbourg, France. FAX: (internat.) + 33 390 24 5140; E-mail: lehn@chimie.u-strasbg.fr

[h] Present address: Dipartimento di Chimica e Chimica Industriale; via Risorgimento, 35, I-56126 Pisa, Italy

[i] Department of Chemistry, University of Toronto, 80 St. George St., M5S3H6, Toronto, ON, Canada

This work is dedicated to Professor Koji Nakanishi, magister of chemistry, teaching, and magic on the occasion of his 80th birthday.

Table of Contents

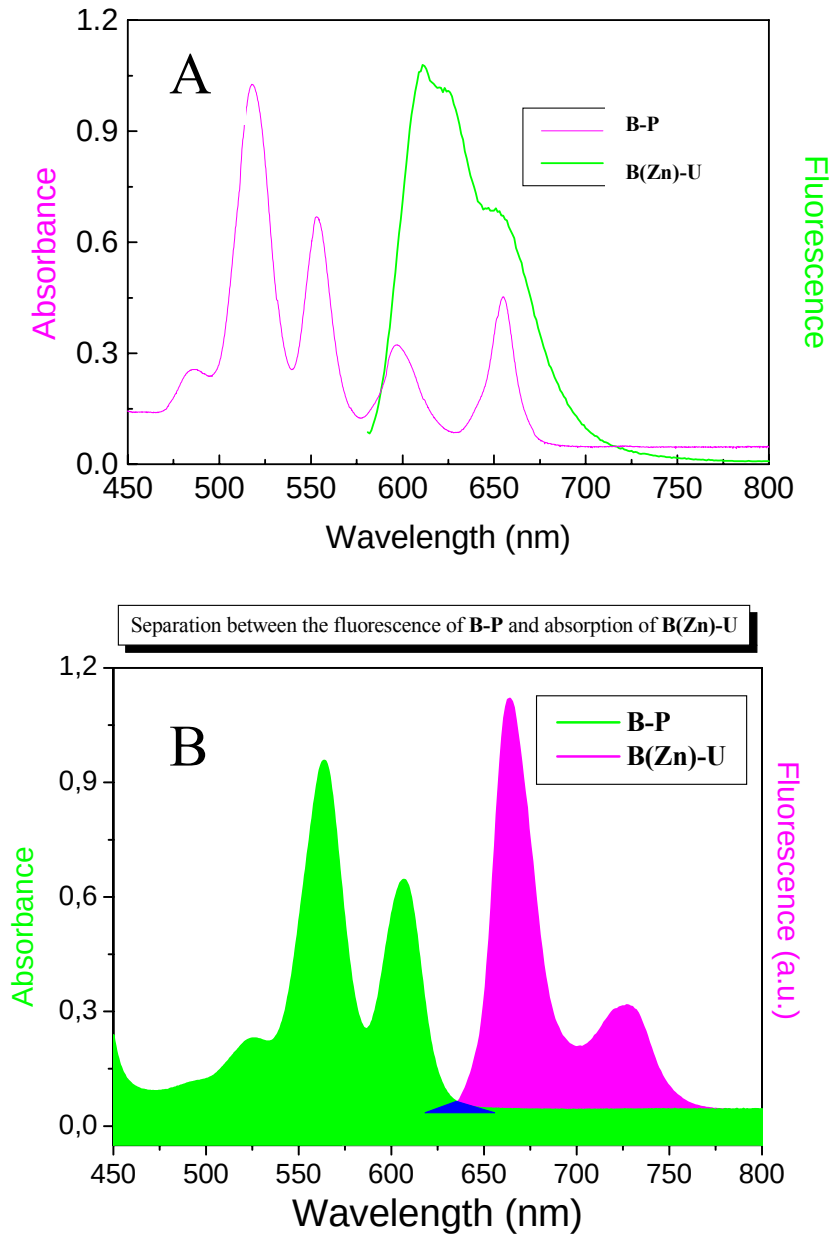


Figure S1. The absorption spectra of the energy acceptor**B-P** overlaps with the emission spectra of the donor **B(Zn)-U** (A, top). Conversely there is minimal overlap between the absorption spectra of **B(Zn)-U** and the emission spectra of **B-P** (B, bottom). Absorption: ~0.3 mM in $CHCl_3$ in 10 mm cuvettes. Fluorescence: ~10 μM in $CHCl_3$ in 10 mm cuvettes, with front face geometry.

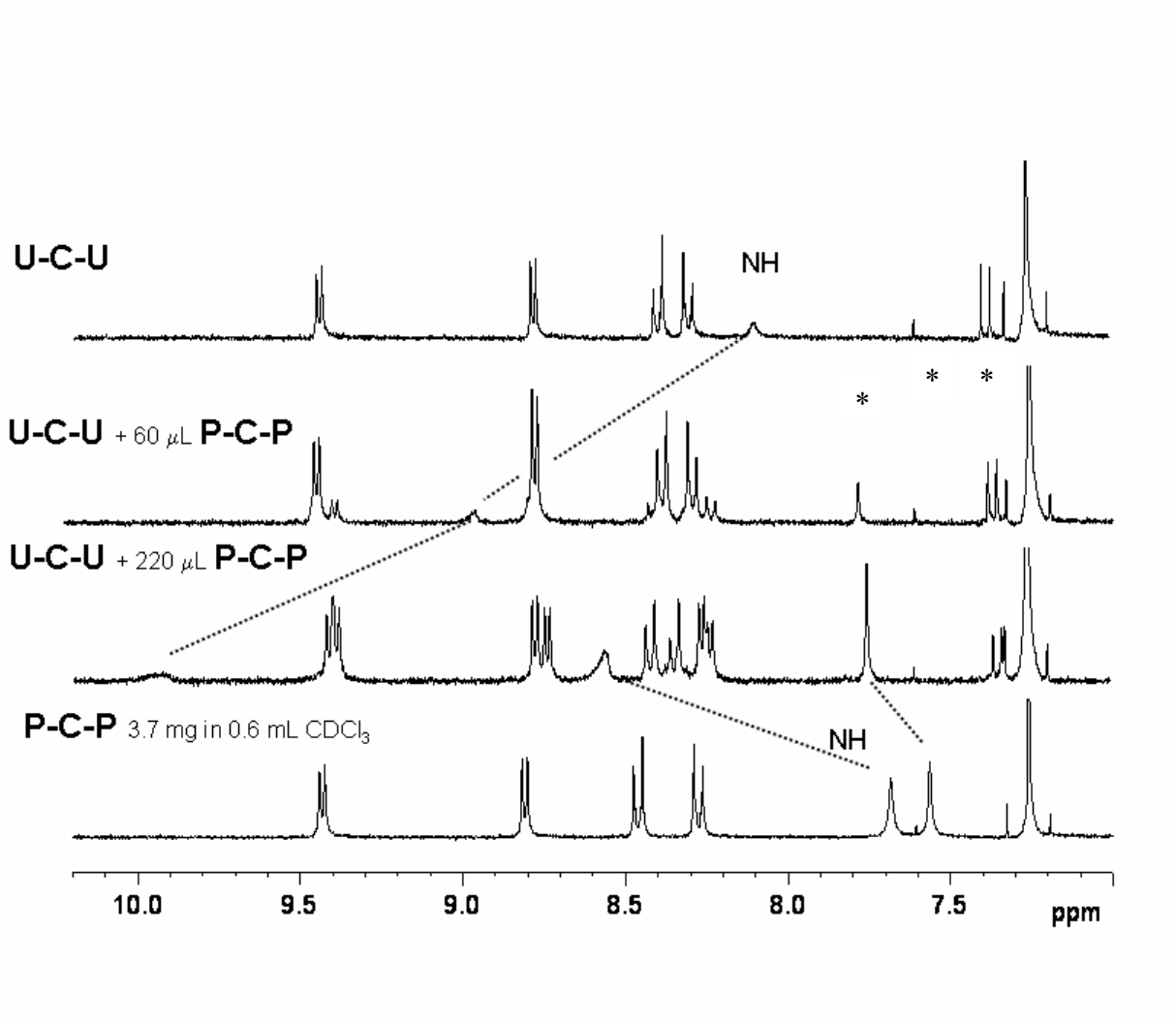


Figure S2. Titration of a solution of **P-C-P** into a solution of **U-C-U**. Low field region of the ^{1}H-NMR spectrum (300MHz). The three small spikes indicated by asterisks flanking the residual chloroform signal (7.26 ppm) are not impurities but due to spinning side bands (20 Hz) and one ^{13}C-satellite band.

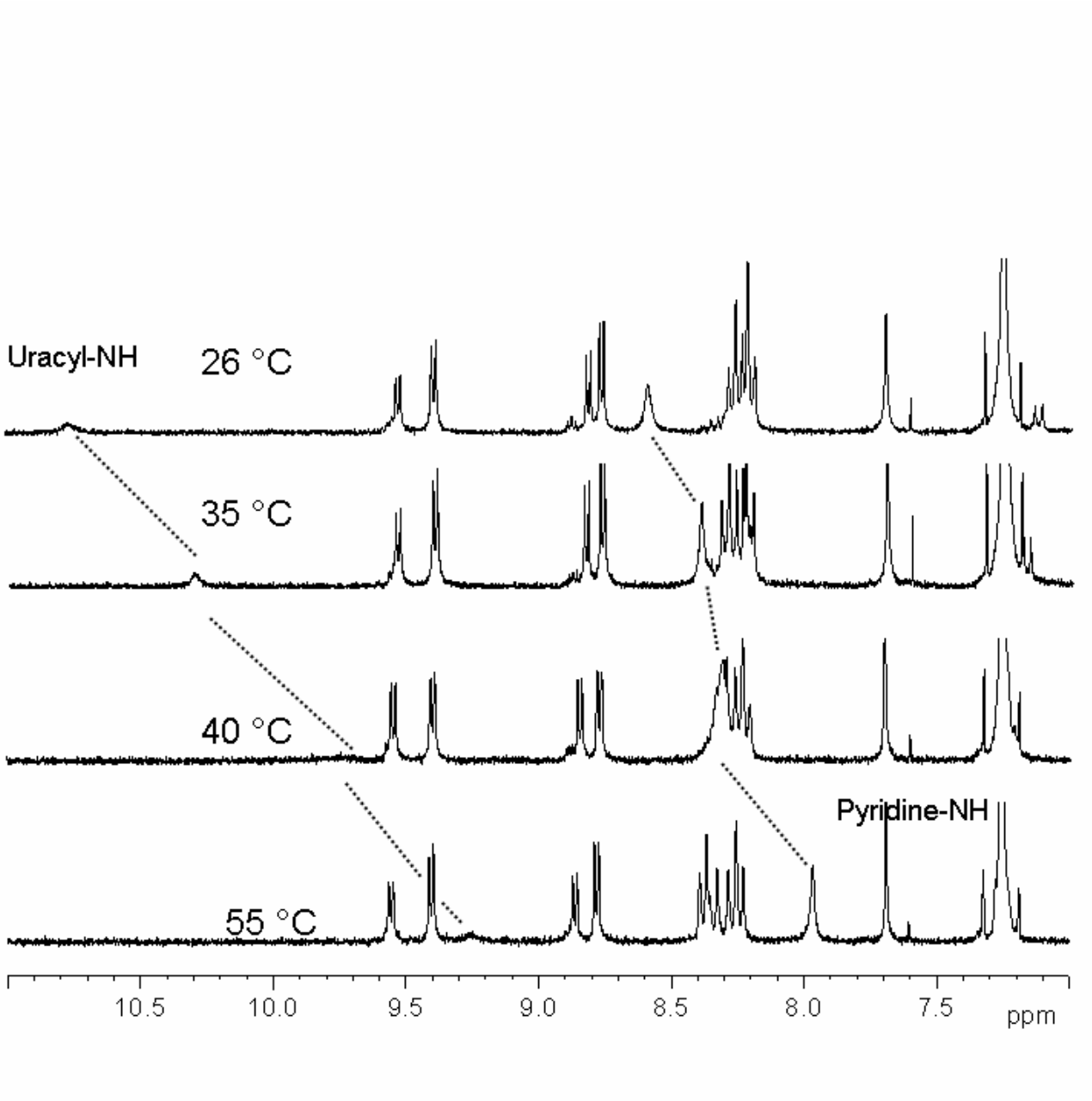


Figure S3. Temperature dependence of a **U-C(Zn)-U / P-C-P** mixture. The sample was heated using the variable temperature unit (± 0.5 °C) and allowing it to equilibrate for 5 minutes before measurement.

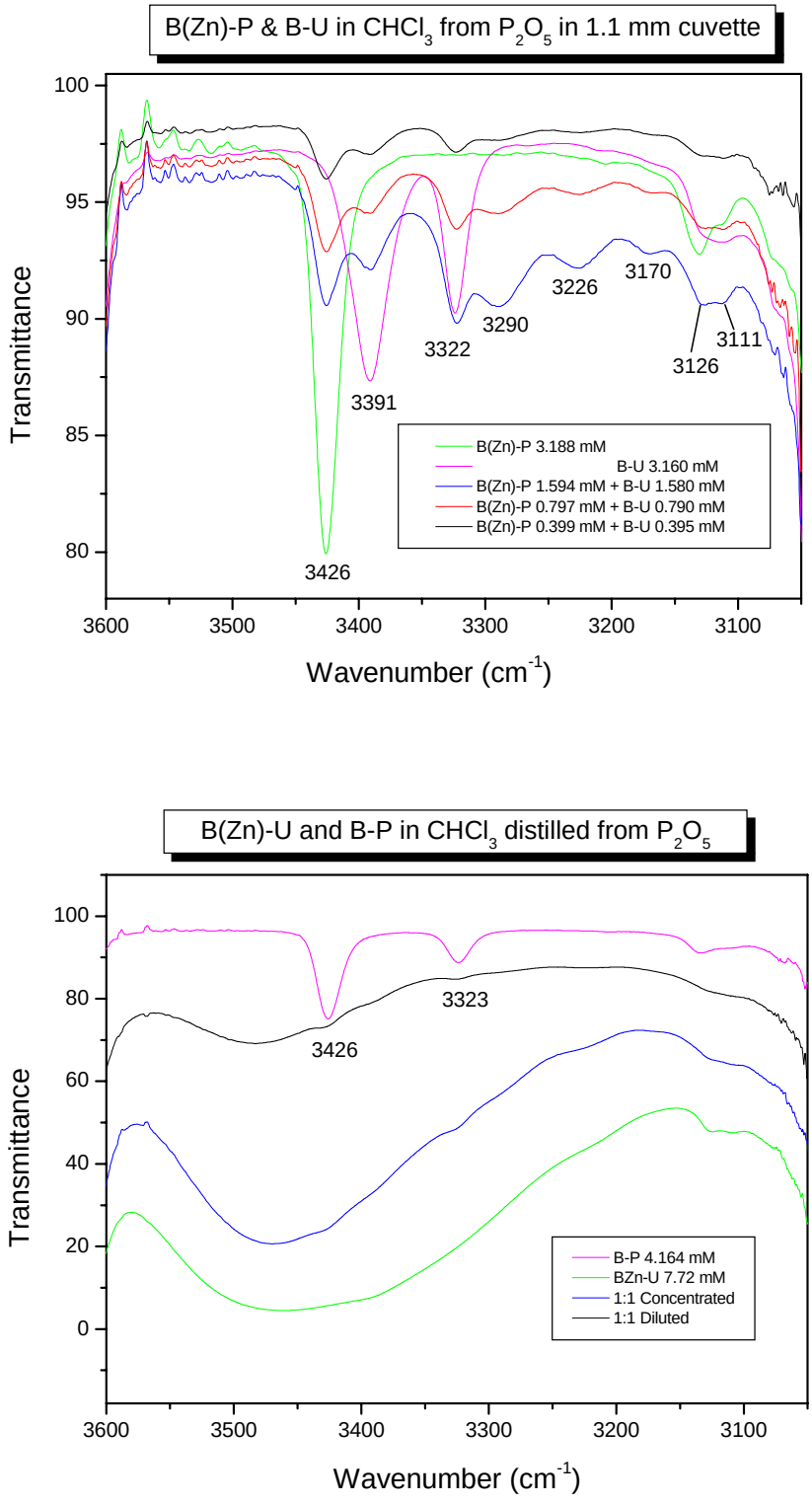


Figure S4. FT-IR Spectra of **B(Zn)-P ≡ B-U** (upper part) and the inverse configuration **B(Zn)-U ≡ B-P** (lower part).

Fluorescence Spectra: Steady State Measuemants

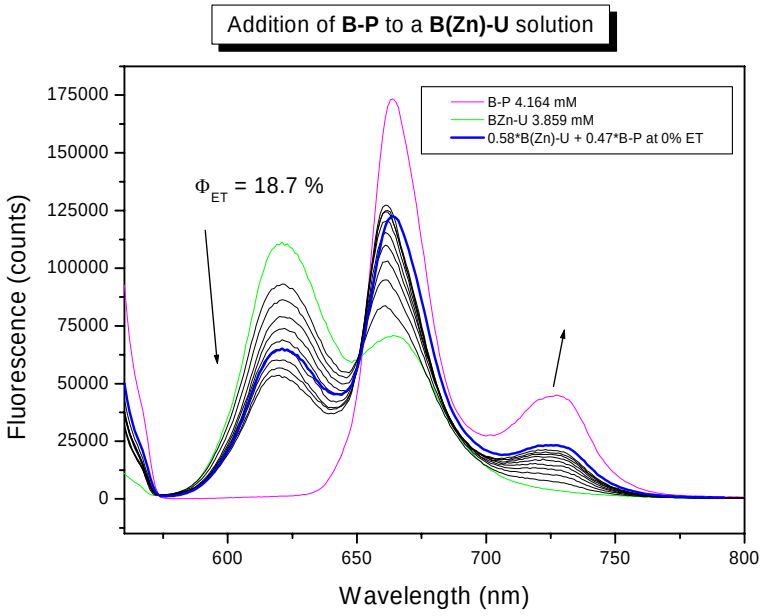


Figure S5. Addition of **B-P** to **B(Zn)-U** in dry chloroform. Note that considerable quenching at 605 nm occurs in comparison with a calculated 1:1 mixture at the same concentrations in the absence of energy transfer (blue line). Excitation was at 550 nm and the measurement was done with a front face geometry.

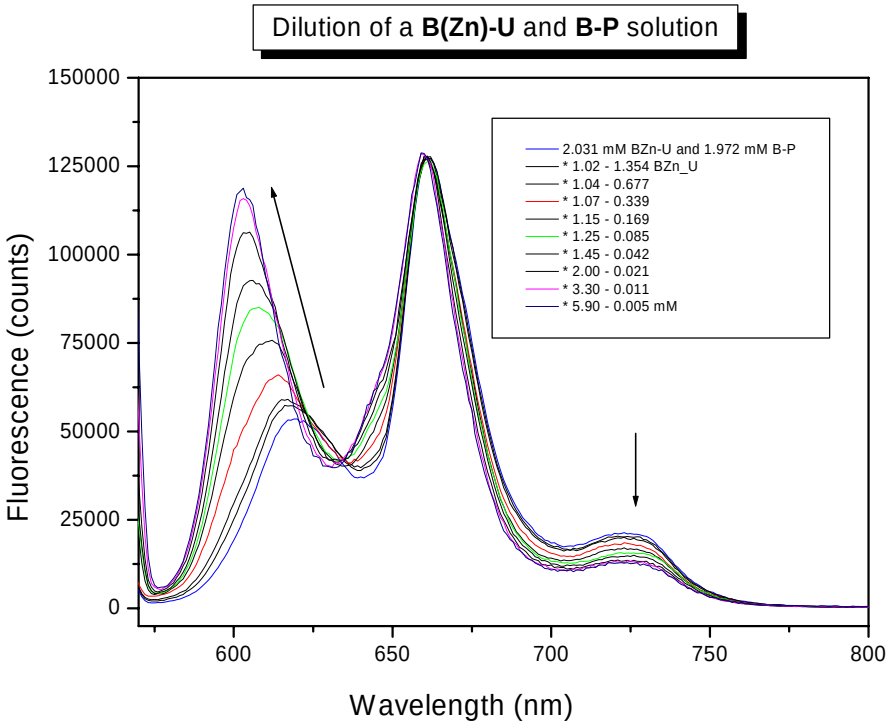


Figure S6. Dilution of a concentrated **B-P** ≡ **B(Zn)-U** mixture with dry chloroform. Note the steep recovery of fluoresce at 605 nm. Excitation was at 550 nm and fluorescence detection was performed using a front face geometry.

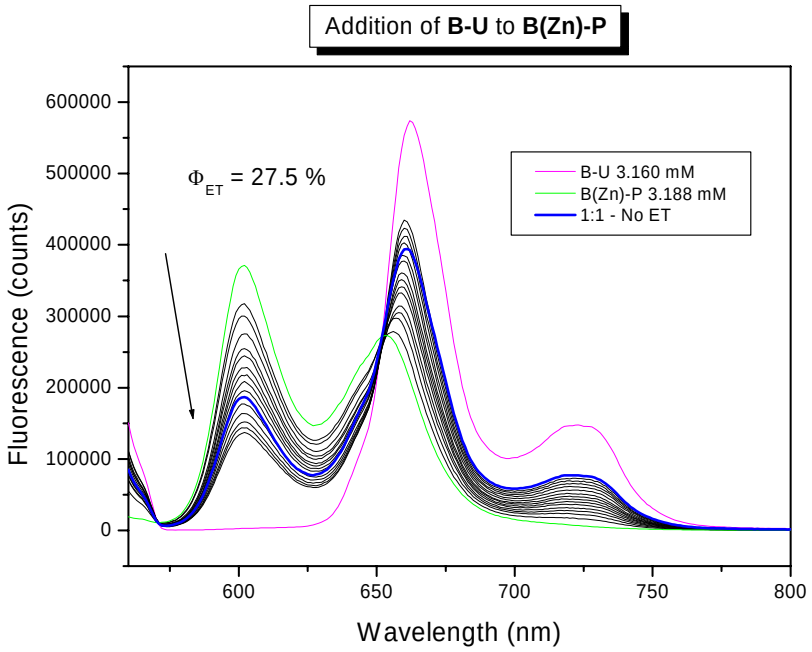


Figure S7. Addition of **B-U** to **B(Zn)-P** in dry chloroform. Note that considerable quenching at 605 nm occurs in comparison with a calculated 1:1 mixture at the same concentrations in the absence of energy transfer (blue line). Excitation was at 550 nm and the measurement was done with a front face geometry.

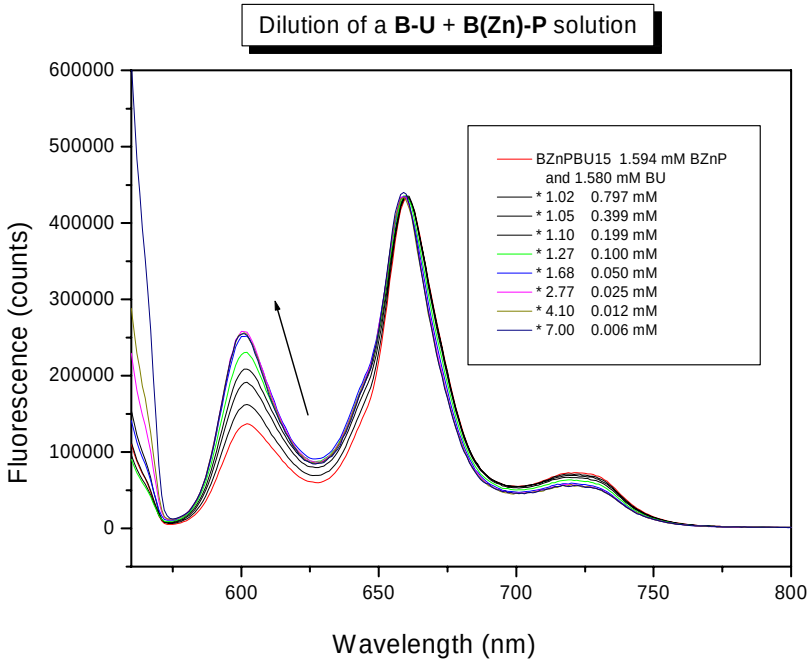


Figure S8. Dilution of a concentrated **B-U ≡ B(Zn)-P** mixture in dry chloroform. Note the steep recovery of fluorescence at 605 nm. Excitation was at 550 nm and fluorescence detection was performed using a front face geometry.

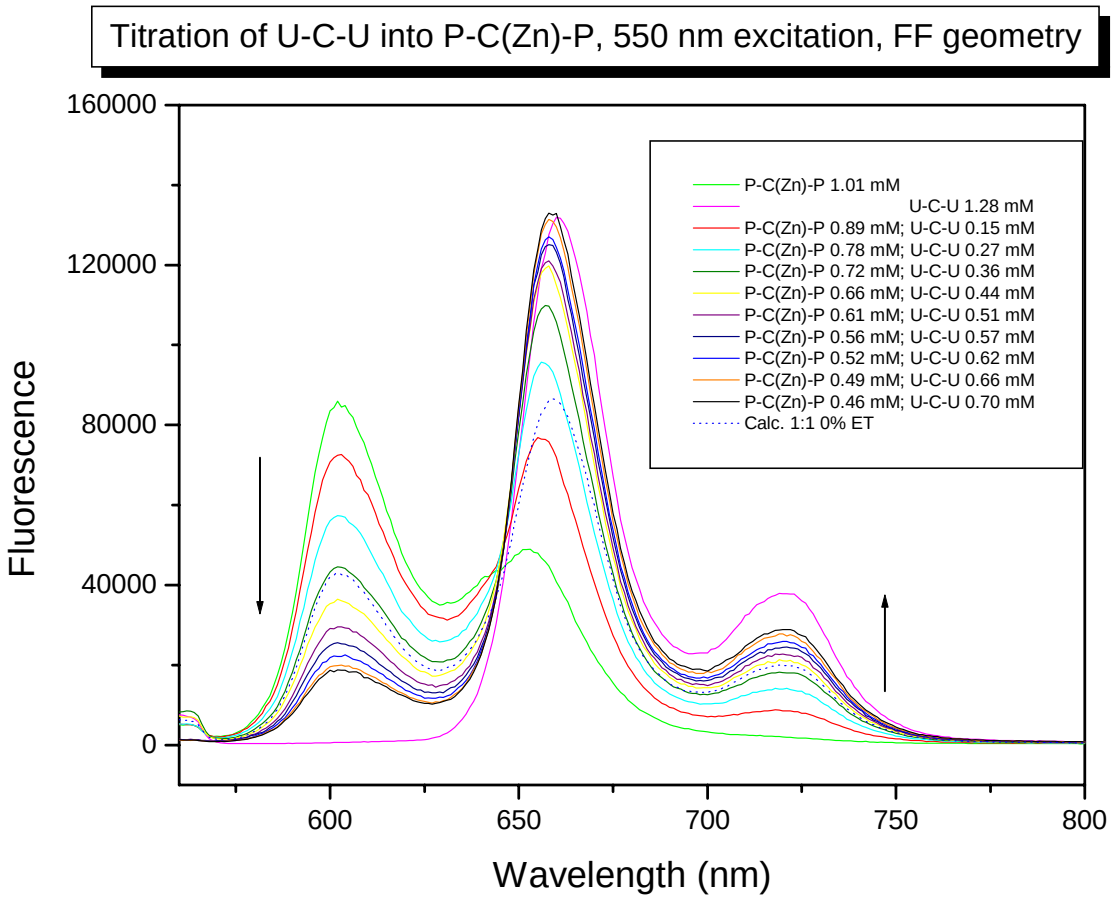


Figure S9 and alternative Figure 2. Addition of **U-C-U** to **P-C(Zn)-P** in dry chloroform. Note that considerable quenching at 605 nm occurs in comparison with a calculated 1:1 mixture at the same concentrations in the absence of energy transfer (dotted line). Excitation was at 550 nm and the measurement was done with front face geometry.

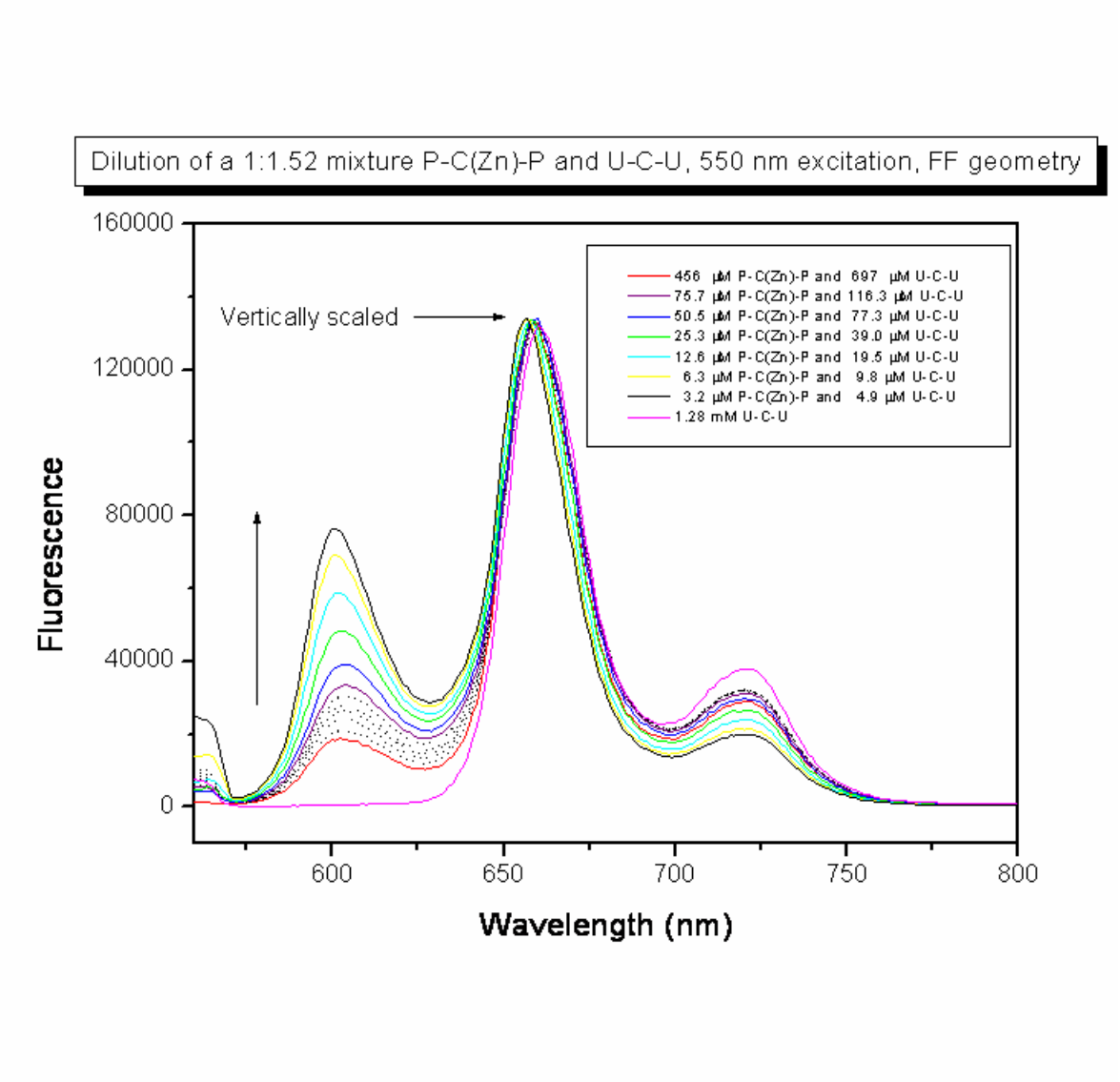


Figure S10. Dilution of a concentrated **P-C(Zn)-P / U-C-U** mixture in dry chloroform. Note the steep recovery of fluorescence at 605 nm. Excitation was at 550 nm and fluorescence detection was performed using a front face geometry.

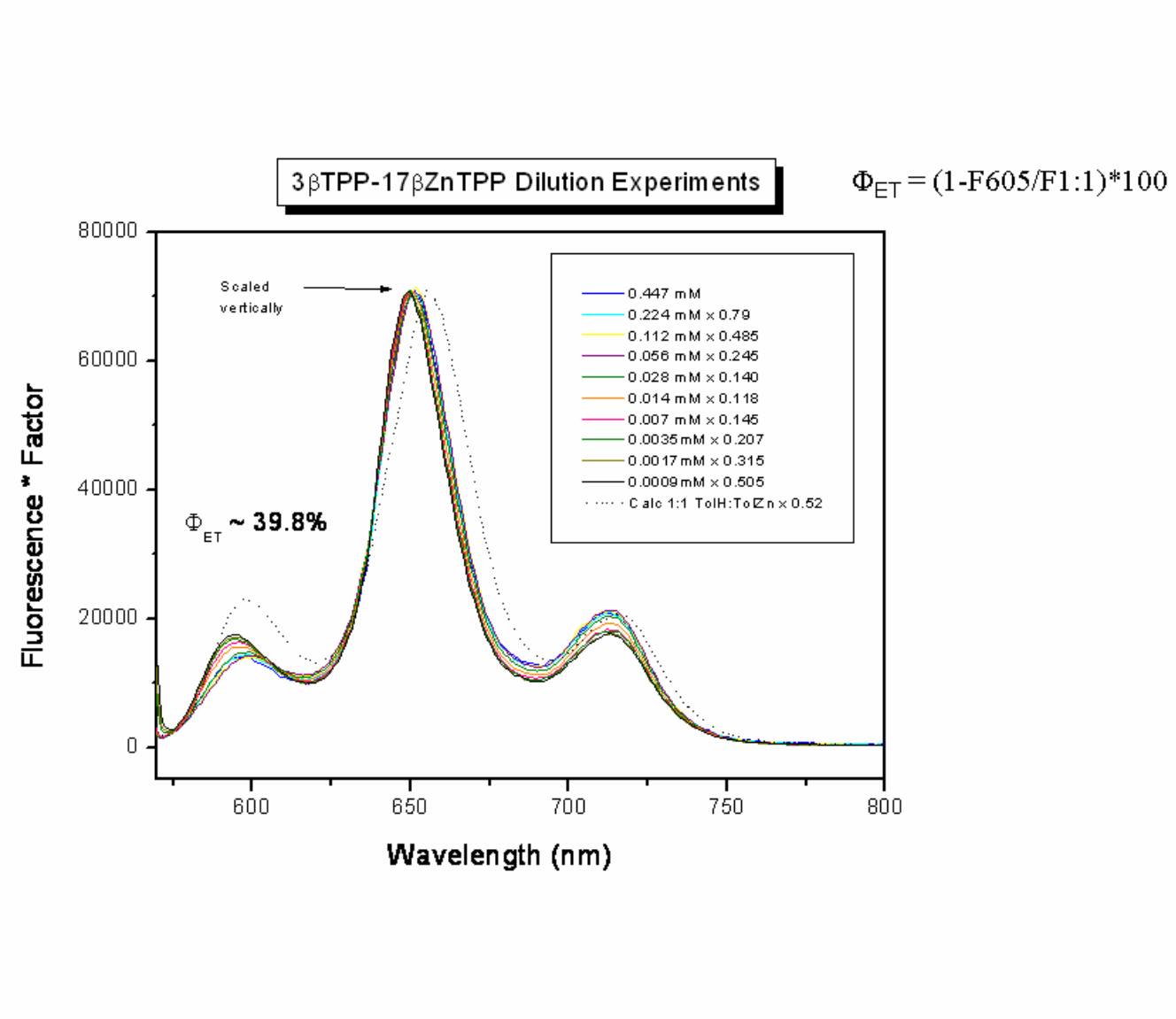


Figure S11. Dilution of a chloroform solution of **3β-17Znβ**. Note that there is no recovery of fluorescence at 602 nm upon dilution over three orders of magnitude (in contrast to Figs. S6, S8 and S10). The dotted line corresponds to a 1:1 **Tol / Tol(Zn)** mixture which shows no energy transfer.

Fluorescence Spectra: Time Resolved Measurements

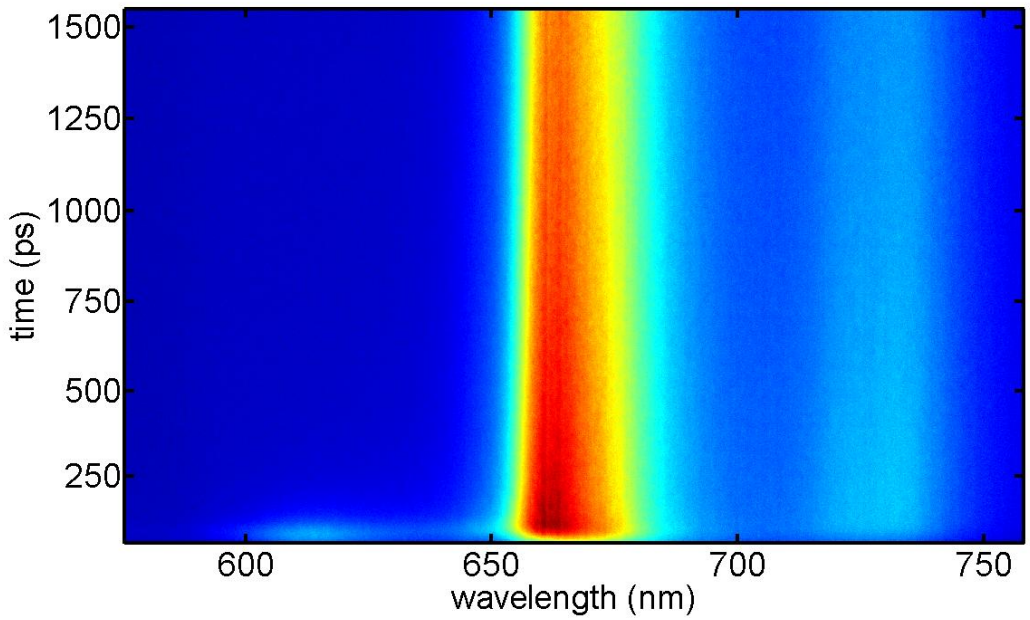


Figure S12a. **B(Zn)-U ≡ B-P** in cyclohexane (~0.05 mM). Note the fast decay of the **B(Zn)U** fluorescence around 610 nm and concomitant rise of the free base at 725 nm. Excitation was at 552 nm, where **B(Zn)-U** absorbs about three times more than the free base **B-P**.

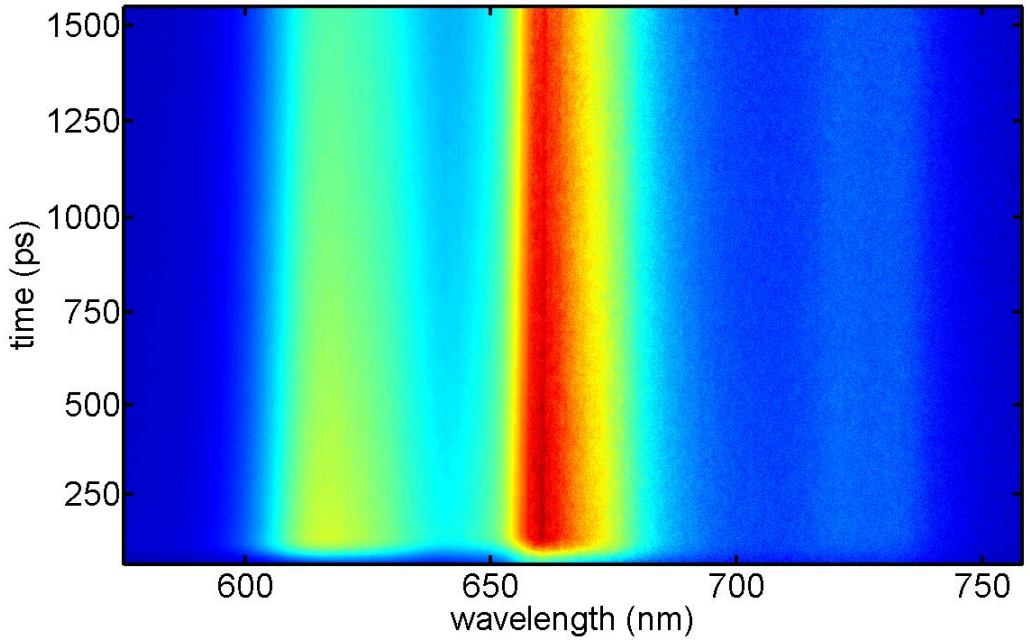


Figure S12b. The same equimolar mixture of **B(Zn)-U ≡ B-P** in dry cyclohexane after addition of a stoichiometric excess of methanol to disrupt the H-bonds.

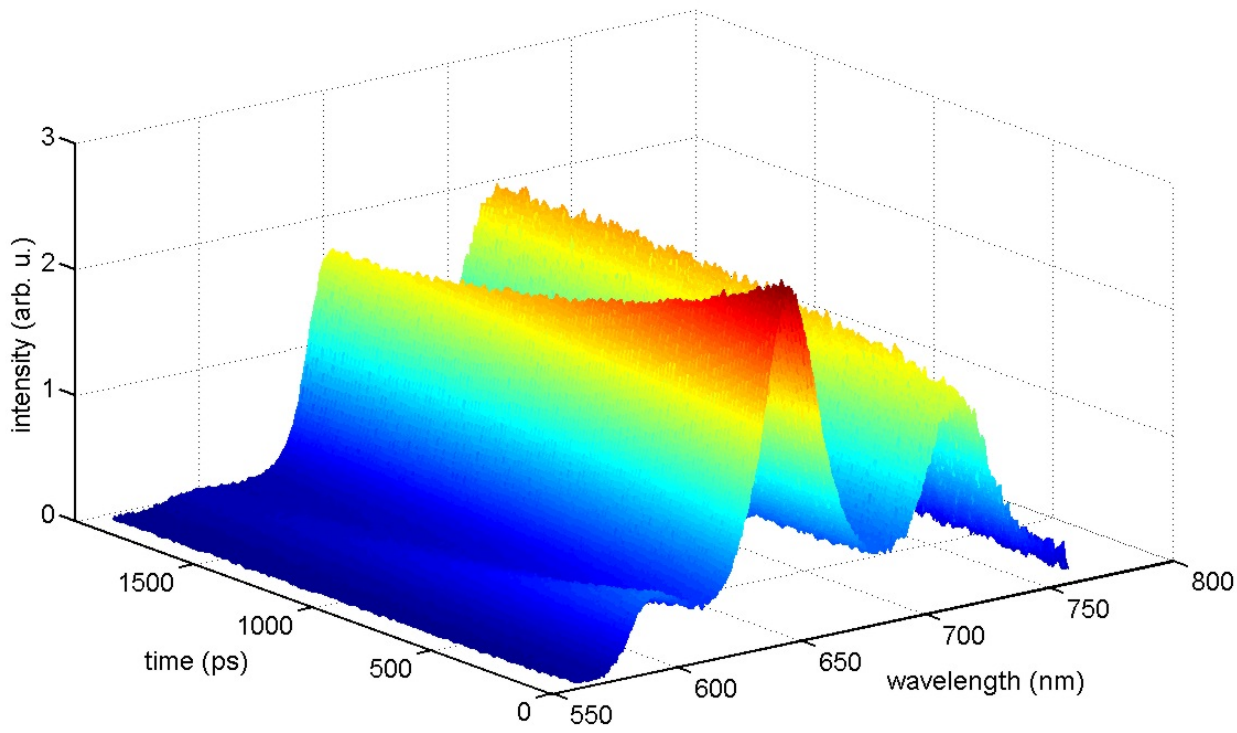


Figure S12c. 3-D plot of the fluorescence decays for **17αZn-3β**. Note the decay of the zinc porphyrin at ca. 610 nm and the rise of the free base component at ca. 740 nm.

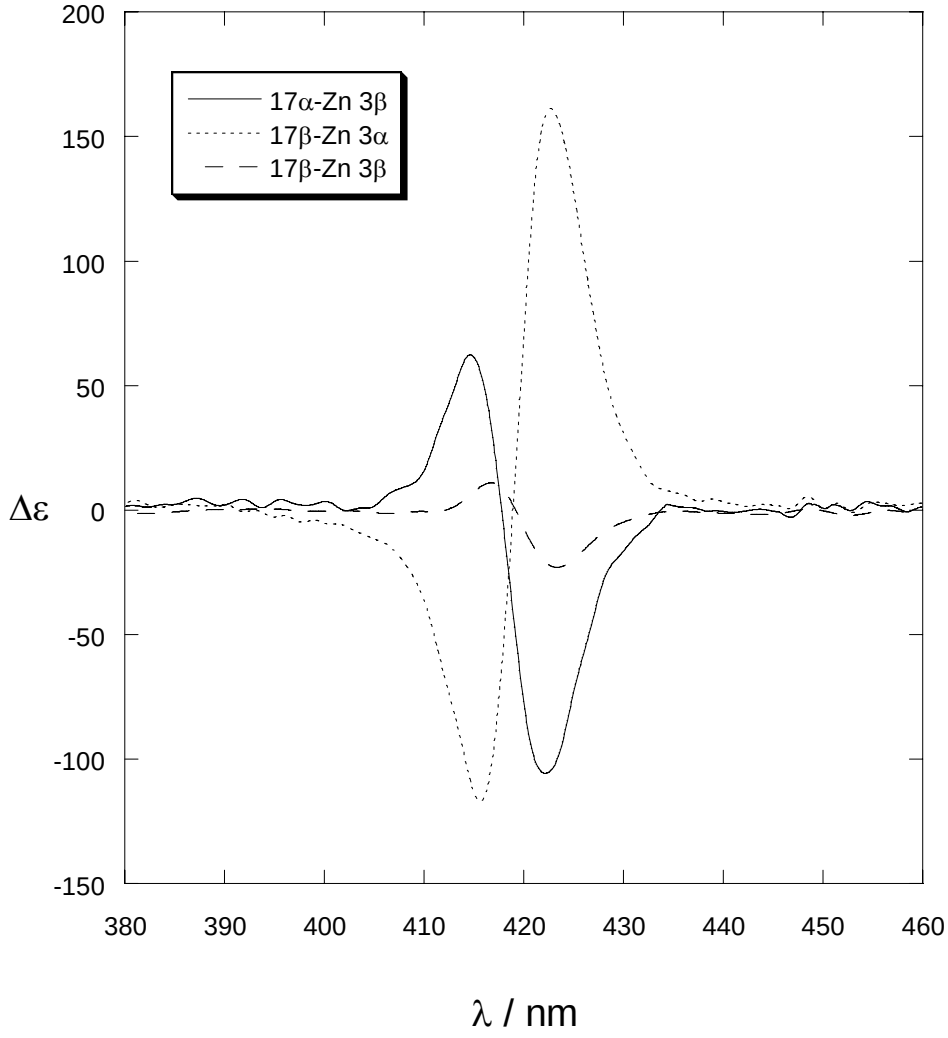


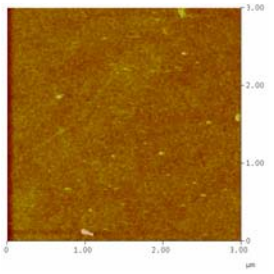
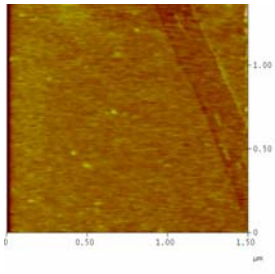
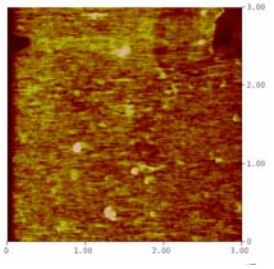
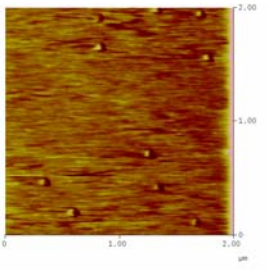
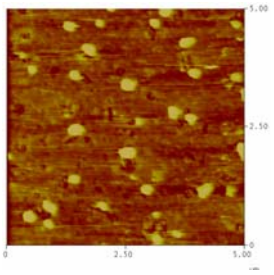
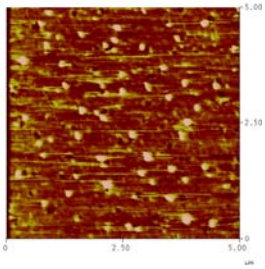
Figure S13. CD spectra of covalent bis-porphyrinic steroids, concentration 5-10 μM measured in a 0.2 cm quartz cell.

Experimental details for the AFM investigations and DLS measurements

Dynamic light scattering (DLS) measurements were recorded using the PD2000DLS (PDDLS/Cool Batch 90T from Precision Detectors) instrument and 0.5 × 0.5 mm glass cuvette, for each individual porphyrin and for the 1:1 mixtures (**UCU**+**PC(Zn)P** and **PCP**+**UC(Zn)U**) at 25μM solution concentrations in chloroform**.** Solutions were filtered through a 0.2 μm syringe filter.

Atomic force microscopy (AFM) images were recorded using a Veco Nanoscope III instrument using contact mode on glass coverslips cleaned using H_2O : NH_4OH : H_2O_2 (4:1:1) followed by several rinses with distilled water and drying in an oven at 100 °C. The 25 μM solution of each individual compound and the 1:1 combinations were drop cast using a 0.2 μm syringe filter onto the clean glass or freshly cleaved mica surface and allowed to dry in the air in a capped cell culture dish. The silicon cantilever (CSC21) contact mode tip had a nominal convolution of 10 nm. Images were taken in several places of the sample where a high, medium, and low density of particles were observed, where the latter two likely are more representative of the aggregates found in solution. Each of the porphyrins and the self-organized aggregates were deposited on at least five different cover slips on different occasions. Several areas of each sample were analyzed by AFM; the histograms represent the particle distribution for the given AFM, but are representative of the other samples.

Atomic Force Microscopy (AFM) of the self-assembled porphyrins.

Compounds	AFM (on glass)	AFM (mica)
PCP		
UC(Zn)U		
PCP+UC(Zn)U		

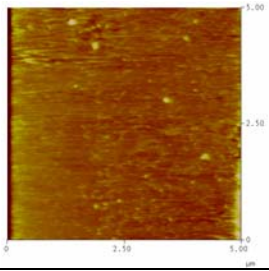
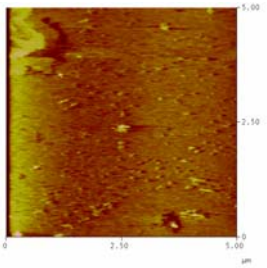
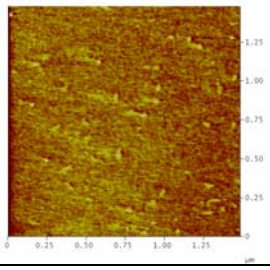
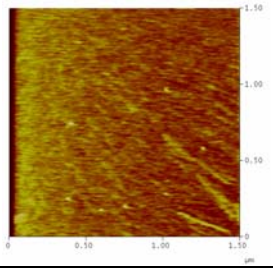
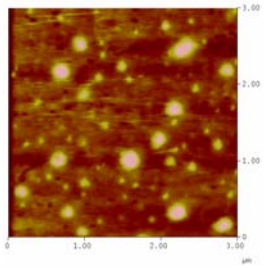
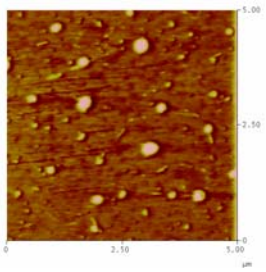
Compounds	AFM (on glass)	AFM (mica)
UCU		
PC(Zn)P		
UCU+PC(Zn)P		

Figure S14. Contact mode atomic force microscopy (AFM) studies of the individual porphyrinic compounds and the self-organized aggregates drop cast onto glass and mica surfaces. The contact mode silicon cantilever (CSC21) tip has a nominal convolution of 10 nm.

Dynamic Light Scattering (DLS) of the self-assembled porphyrins

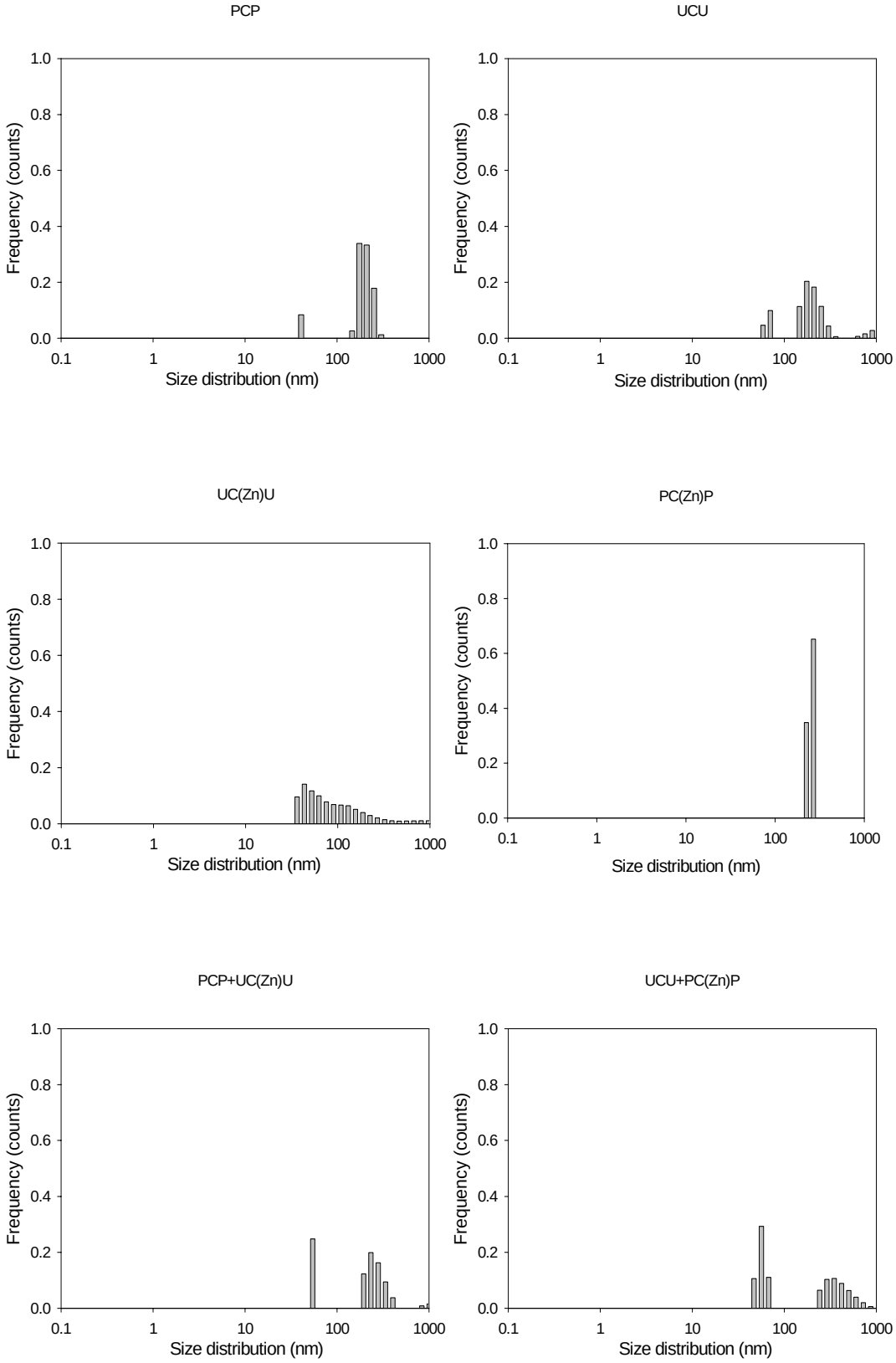


Figure S15. Histograms from the Dynamic Light Scattering (DLS) data. For experimental conditions see above.

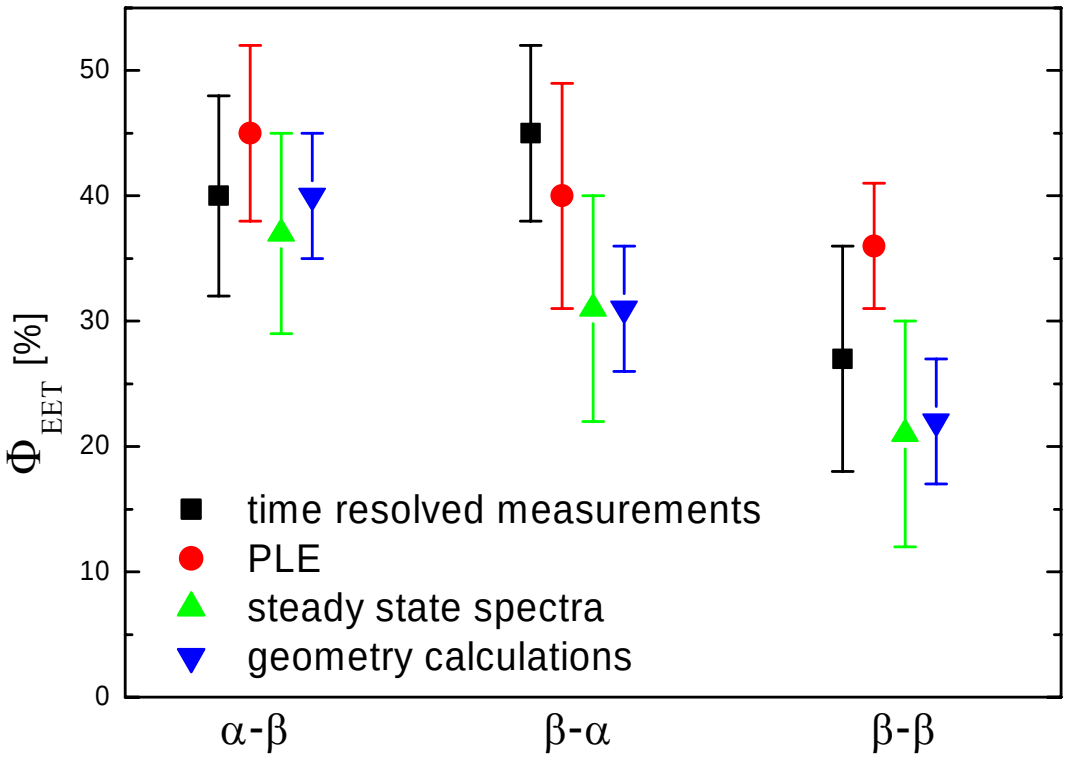


Figure S16. Comparison between the calculated and experimentally determined EET efficiencies.

Experimental Part

General methods. Chloroform and $CDCl_3$ were dried by boiling overnight with P_2O_5 and were freshly distilled before spectroscopic measurements. Cyclohexane was dried by distillation from sodium. Melting points were measured with a Büchi B-540 apparatus in open capillaries and are not corrected. NMR spectra were recorded at 200 MHz (^{1}H) and 50.33 MHz (^{13}C) on a Bruker AC 200 machine and at 300 MHz (^{1}H) on a Bruker DPX 300 Avance instrument. Chemical shifts are given in ppm and were calibrated to the residual non-deuterated signal of the solvent. For $CHCl_3$ $\delta = 7.26$ (for ^{1}H) and 77.0 for ^{13}C. Absorption spectra were measured on a Varian Cary 3 and a Varian Cary 500 spectrometer equipped with a Peltier variable temperature unit. Stationary fluorescence spectra were measured on Jobin-Yvon and Cary Eclipse fluorimeteres. Details of the time-resolved fluorescence measurements are given in the main text. FT-IR spectra were measured on Perkin-Elmer spectrometers (either 1600 or Spectrum GX). FAB mass spectra were obtained from *o*-nitrobenzyl alcohol matrix by the Service de Spectrométrie de Mass de l'Université Louis Pasteur in Strasbourg by means of a Micromass Autospec instrument. Alternatively, MALDI-ToFF spectra were obtained on a Biosystems Voyager instrument using dithranol (1,8,9-anthracenetriol) as matrix. Elemental Analyses were obtained either at the Service de Microanalyse de l'Université Louis Pasteur in Strasbourg or at the Institute for Nanotechnology (Karlsruhe) using a Carlo Erba CEA – Flash microanalysator. Retention factors were measured on silica gel TLC plates from Macherey – Nagel which were eluted with dichloromethane stabilized with 0.2 % ethanol. Column chromatography was performed on Merck silica gel (40-63 μm).

Compounds and abbreviations: steroidal compounds

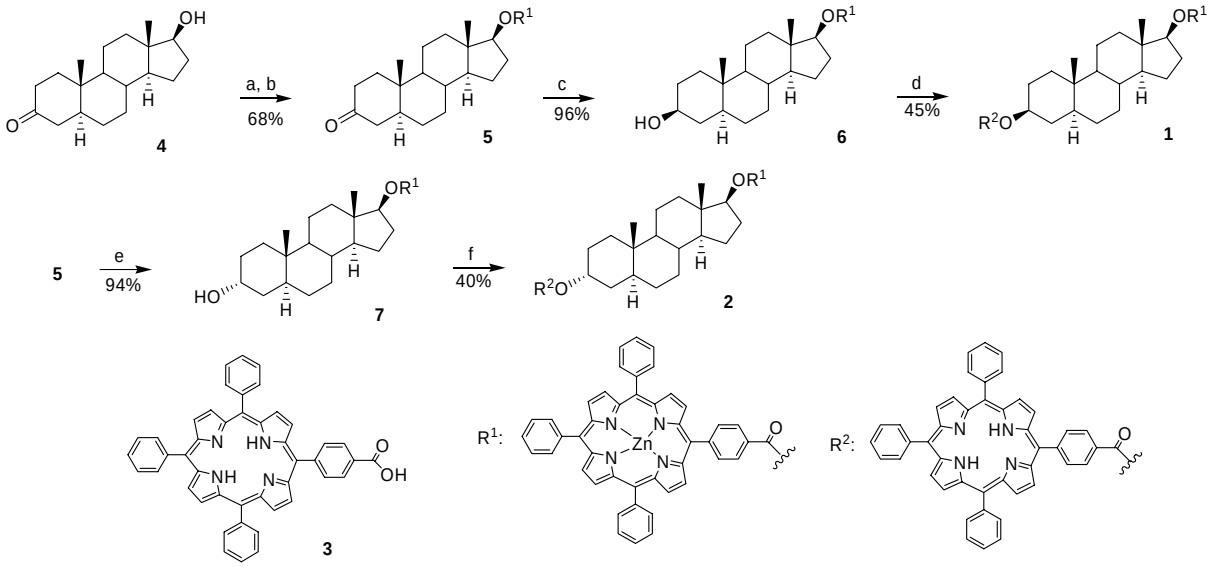
Full name		Short abbr.
5α-Androstane-3β-ol-(*p*-(10',15',20'-triphenyl-5'-porphyrinyl)benzoate-17β-ol-(*p*-(10',15',20'-triphenyl-5'-zinc-porphyrinyl)benzoate)		**3β - 17β**
5α-Androstane-3α-ol-(*p*-(10',15',20'-triphenyl-5'-porphyrinyl)benzoate-17β-ol-(*p*-(10',15',20'-triphenyl-5'-zinc-porphyrinyl)benzoate)		**3α - 17β**
5α-Androstane-3β-ol-(*p*-(10',15',20'-triphenyl-5'-porphyrinyl)benzoate-17α-ol-(*p*-(10',15',20'-triphenyl-5'-zinc-porphyrinyl)benzoate)		**3β – 17α**

Compounds and abbreviations: compounds with hydrogen bond motifs

Full name		Short abbr.
Ester of *N*1-(2-Hydroxy-1-ethyl)-uracyl with 5-(4-carboxyphenyl)-10,15,20-triundecyl-porphyrin		**B-U**
Ester of *N*1-(2-Hydroxy-1-ethyl)-uracyl with 5-(4-carboxyphenyl)-10,15,20-triundecyl-porphyrinato zinc(II)		**B(Zn)-U**
Ester of 2,6-diacetamido-4-(2-Hydroxy-1-ethyl)-pyridine with 5-(4-carboxyphenyl)-10,15,20-triundecyl-porphyrin		**B-P**
Ester of 2,6-diacetamido-4-(2-Hydroxy-1-ethyl)-pyridine with 5-(4-carboxyphenyl)-10,15,20-triundecyl-porphyrinato zinc(II)		**B(Zn)-P**
Diester of *N*1-(2-Hydroxy-1-ethyl)-uracyl with 5,15-bis-(4-carboxyphenyl)-10,20-diundecyl-porphyrin		**U-C-U**

Diester of *N*1-(2-Hydroxy-1-ethyl)-uracyl with 5,15-bis-(4-carboxyphenyl)-10,20-diundecyl-porphyrinato zinc(II)		**U-C(Zn)-U**
Diester of 2,6-diacetamido-4-(2-Hydroxy-1-ethyl)-pyridine with 5,15-bis-(4-carboxyphenyl)-10,20-diundecyl-porphyrin		**P-C-P**
Diester of 2,6-diacetamido-4-(2-Hydroxy-1-ethyl)-pyridine with 5,15-bis-(4-carboxyphenyl)-10,20-diundecyl-porphyrinato zinc(II)		**P-C(Zn)-P**

SI - Scheme I.



Reagents and conditions: a) **3**, EDC, DMAP, CH_2Cl_2, rt, 12 h; b) $Zn(OAc)_2$, MeOH, CH_2Cl_2, rt, 12 h; c) $NaBH_4$, THF, rt, 3h; d) **3**, EDC, DMAP, CH_2Cl_2, rt, 12 h; e)L-selectride, THF, rt, 3h; f) **3**, EDC, DMAP, CH_2Cl_2, rt, 12 h.

5α-Androstane-3β-ol-(*p*-(10',15',20'-triphenyl-5'-porphyrinyl)benzoate-17β-ol-(*p*-(10',15',20'-triphenyl-5'-zinc-porphyrinyl)benzoate) 1. To a solution of porphyrin acid **3** (50 mg, 0.11 mmol), EDC (100 mg, 0.52 mmol), and DMAP (20 mg, 0.18 mmol) in anhydrous CH_2Cl_2 (3 mL) was added androstane **6** (21 mg, 23 μmol). The mixture was stirred at room temperature for 12 hours, and diluted with CH_2Cl_2 (10 mL), extracted with a solution of saturated NH_4Cl (20 mL) and dried with Na_2SO_4. Flash column chromatography gave the desired product **1** in 45 % yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.74 – 9.06 (m, 16H), 8.40 - 8.48 (m, 4H), 8.28 - 8.35 (m, 4H), 8.15 – 8.25 (m, 6H), 8.16 - 8.28 (m, 12H), 7.72 – 7.82 (m, 18H), 5.10 – 5.15 (m, 1H), 5.00 (t, 1H, *J* = 8.3 Hz), 0.81 – 2.52 (m, 22H), 1.14 (s, 3H), 1.04 (s, 3H), -2.79 (bs, 2H). FAB-HRMS *m/z* for $C_{109}H_{86}O_4N_8Zn$ calc. 1634.6063, found 1634.6051. CD (CH_2Cl_2): 423 nm (-12), 414 nm (+11).

5α-Androstane-3α-ol-(*p*-(10',15',20'-triphenyl-5'-porphyrinyl)benzoate-17β-ol-(*p*-(10',15',20'-triphenyl-5'-zinc-porphyrinyl)benzoate) 2. To a solution of porphyrin acid **3** (50 mg, 0.11 mmol), EDC (100 mg, 0.52 mmol), and DMAP (20 mg, 0.18 mmol) in anhydrous CH_2Cl_2 (3 mL) was added androstane **6** (21 mg, 23 μmol). The mixture was stirred at room temperature for 12 hours, and diluted with CH_2Cl_2 (10 mL), extracted with a solution of saturated NH_4Cl (20 mL) and dried with Na_2SO_4. Flash column chromatography gave the desired product **2** in 40 % yield. ^{1}H-NMR (400 MHz, C_6D_6) δ 9.15 (d, 2H, *J* = 4.7 Hz), 9.08 (bs, 4H), 8.90 – 9.05 (m, 8H), 8.83 (d, 2H, *J* = 4.7 Hz), 8.61 (d, 2H, *J* = 8.0 Hz), 8.48 (d, 2H,

J = 8.0 Hz), 8.05 - 8.24 (m, 17H), 7.40 – 7.55 (m, 18H), 5.48 – 5.58 (m, 1H), 5.00 (t, 1H, *J* = 8.3 Hz), 0.81 – 2.52 (m, 22H), 1.05 (s, 3H), 0.69 (s, 3H), -2.12 (bs, 2H). FAB-HRMS *m/z* for $C_{109}H_{86}O_4N_8Zn$ calc. 1634.6063, found 1634.6046. CD (CH_2Cl_2): 422 nm (+139), 413 nm (-87).

Details of the other precursors can be found in the PhD Thesis of Xuefei Huang, Columbia University, New York, or from the authors.

1-(2-Hydroxyethyl)-uracyl. The glassware was dried in the oven (110 °C) overnight, was assembled while hot and was left to cool under argon. Uracyl (Aldrich) was dried for 4.5 hrs at 120-130 °C at the vacuum pump. DMF was stirred overnight with barium oxide and was freshly distilled in vacuum before use. Uracyl (15.0 g) was reacted with ethylene carbonate (Aldrich, 13.2 g) in DMF (300 mL) in the presence of sodium hydroxide (two quickly grinded pellets). At 140 °C the reaction mixture goes into solution. It was heated to 155 °C (bath temperature with gentle reflux) for one hour; then the heating was stopped and the mixture was left overnight under magnetical stirring. The clear pale yellow solution was then heated on a rotary evaporator for two hours. As the bath temperature reached 80 °C, DMF started to distil slowly. In the end, to the solidified reaction mass 400 mL distilled water were added. The initially thick precipitate becomes very fine upon rubbing with a rounded spatula. This unreacted uracyl was filtered on a Buchner funnel and the pale yellow filtrate had a pH of 8.0. The precipitate was washed once with 10 mL of distilled water, dried, weighed (1.69 g) and discarded. To the filtrate, strongly acidic DOWEX 50 resin was added (three spatula) and after gentle shaking, it was filtered through a folded paper filter. After evaporation of the water on a rotary evaporator and subsequently on the vacuum pump, 20.64 g of crude N-hydroxyethylated derivatives were left. The chromatographic separation of the desired 1-(2-hydroxyethyl)- and of the 1,3-bis-(2-hydroxyethyl)-uracyl was somewhat problematic. While on a TLC plate with various eluents the mono- and the bis-*N*-alkylated products are virtually indistinguishable having almost the same R_f (best separation was achieved with $CHCl_3$/MeOH = 4/1), a neat separation could be performed on a silica gel column eluted with chloroform to which gradually increasing amounts of methanol are added. Thus, 2.58 g of crude reaction mixture were deposited from hot methanol onto 6.16 g of silicagel. This was added to the top of a 22 cm column (Φ = 4 cm). The bis-*N*-alkylated product is eluted first and as the eluent reaches 3% methanol in chloroform, the desired 1-(2-hydroxyethyl)-uracyl is eluted. Monitoring of the eluent proved to be possible by ^{1}H-NMR. The yield of separated, purified product was 21.4 %. The pure dihydroxyethyluracyl was isolated in 14 % yield based on uracyl. The rest were either mixed or impure fractions. This procedure is an adaptation of the procedures described in the following two references :
[1] H.-J. Gi, Y. Xiang, R.F. Schinazi and K. Zhao, *J. Org. Chem.* **1997**, *62*, 88-92.
[2] J. Pitha and P. O. P. Ts'o, *J. Org. Chem.* **1968**, *33*, 1341-1344.

Esterification with porphyrinic free acid B to B-U

In an oven-heated, two-necked 50 mL flask equipped with a magnetical stirrer, rubber septum and argon bubbler, after argon purging and cooling, are placed as solids after weighing, the vacuum dried acid B-COOH (123.7 mg, 0.138 mmol), DMAP (16.9 mg, 0.138 mmol) and EDCI (26.5 mg, 0.138 mmol). Dichloromethane (about 10 mL), freshly distilled from CaH_2 under argon, is cannulated via the septum and stirring is continued until complete dissolution of the solids. 1-(2-Hydroxyethl)-uracyl (21.6 mg, 0.138 mmol) vacuum dried, is suspended in the argon-purged oven-heated vial in which it was weighed, in dichloromethane. It is then

charged into a 10 mL syringe and is added slowly (over 5 min.) to the reaction mixture. The vial and the syringe are rinsed once with additional dichloromethane. The total volume of dichloromethane added to the reaction mixture was 20 mL. The reaction mixture is protected from light and stirred for 48 hrs at room temperature. The reaction mixture is then diluted with argon degassed dichloromethane (125 mL) and washed three times with saturated aqueous ammonium chloride and then twice with saturated sodium chloride solution. After drying over sodium sulphate, the solvent is removed in vacuum leaving 163.3 mg of crude reaction product (yield = 117 %) which according to TLC contains mainly the desired ester in addition to a very fast moving band and a much slower one. Recrystallization from hot ethyl acetate and cooling overnight at –18 °C affords 92.8 mg of pure ester (yield = 66.5 %). The filtrate could be concentrated in vacuum and conveniently chromatographed on silica gel (H = 12 cm, Φ = 2,2 cm) eluted with dichloromethane : methanol = 98 : 2 giving an additional amount of 27 mg pure ester B-U, global yield = 86 %.
This procedure consists of an adaptation from the following references :

[3] S. Matile, N. Berova, K. Nakanishi, S. Novkova, I. Philipova and B. Blagoev, *J. Am. Chem. Soc.* **1995**, *117*, 7021-7022.
[4] S. Matile, N. Berova, K. Nakanishi, J. Fleischhauer and R. W. Woody, *J. Am. Chem. Soc.* **1996**, *118*, 5198-5206.
[5] M. K. Dhaon, R. K. Olsen and K. Ramasamy, *J. Org. Chem.* **1982**, *47*, 1962-1965.
[6] P. S. Baran, R. R. Monaco, A. U. Khan, D. I. Schuster and S. R. Wilson, *J. Am. Chem. Soc.* **1997**, *119*, 8363-8364.

R_f = 0.58 (on SiO_2 eluted with CH_2Cl_2 : MeOH = 95 : 5).

^{1}H-NMR (200 MHz, $CDCl_3$) : 9.48 (4H, sharp quartet, 12-, 13-, 17-, 18-H) 9.38 (2H, d, J = 4.7 Hz, 2-, 8-H), 8.75 (1H, broad, 3"-NH), 8.72 (2H, d, J = 4.9 Hz, 3-, 7-H), 8.34 (2H, d, J = 8.5 Hz, 3'-, 5'-H), 8.25 (2H, d, J = 8.5 Hz, 2'-, 6'-H), 7.24 (1H, d, partially obscured by the solvent peak, 6"-H), 5.77 (1H, d, J = 7.9 Hz, 5"-H), 4.93 (6H, m, 1^{IV}-CH_2), 4.72 (2H, broad t, OCH_2), 4.17 (2H, broad t, NCH_2), 2.51 (6H, quintet, 2^{IV}-CH_2), 1.79 (6H, quintet, 3^{IV}-CH_2), 1.52 (6H, m, 4^{IV}-CH_2), 1.27 (36H, s, 5^{IV} to10^{IV}-CH_2), 0.90 (9H, t, 11^{IV}-CH_3), -2.66 (2H, s, NH).

^{13}C-NMR (50 MHz, $CDCl_3$): 166.31 (COO), 163.26 (4"), 150.62 (2"), ~146.5 (very broad C_α-pyrrole, n.v.), 144.66 (1', 6"?), 134.66 (2'-, 6'-C), ~130.8 (broad, 3-, 7-C, n.v.), 128.27 (4'-C), ~128.0 (broad, 2-, 8-, 12-, 13-, 17-, 18-C), 127.76 (3'-, 5'-C), 120.04 (5-C), 119.49 (10, 15, 20-C), 102.36 (5"-C), 62.41 (OCH_2), 48.07 (NCH_2), 38.74 (2^{IV}-CH_2), 35.43 (1^{IV}-CH_2), 31.87 (9^{IV}-CH_2), 30.65 (3^{IV}-CH_2), 29.70, 29.62 and 29.33 (4^{IV} to 8^{IV}-CH_2), 22.68 (10^{IV}-CH_2), 14.12 (11^{IV}-CH_3). n.v. = not visible at the registered number of scans.

FAB-MS: 1031.9 (M+H)+, 889.7 (M+H-$C_{10}H_{21}$)+.
HR FAB-MS: 1031.7059 found for (M+H)+ ;
1031.7102 calculated for $C_{66}H_{91}N_6O_4$.

IR (KBr): 3315 w (NH), 2922 s, 2861 s, 1727 and 1686 s (C=O), 1606 w, 1458 m, 1269 s (O-CH2), 1112 m, 790 s, 732 m.
UV-Vis (CH_2Cl_2), λ_{max} (lg ε_{max}): 655 (3.87), 597 (3.74), 554 (4.07), 518 (4.23), 418 (5.61), 260 (4.41).
$C_{66}H_{91}N_6O_4$: Calcd. C: 76.85; H: 8.79; N: 8.15. Found: C: 76.56; H: 8.70; N: 8.02.

Zinc metalation to B(Zn)-U. General Procedure.

The filtrate from the recrystallization of (**B-U**) (28.5 mg) was dissolved in dichloromethane and then methanol was added until the solution clouded. Excess zinc acetate dihydrate was added and then the mixture was degassed by argon bubbling and stoppered. After stirring for 1.5 hours at room temperature, dichloromethane was added and the methanol and zinc salt were washed away by shaking twice with aqueous sodium hydrogen carbonate and then with brine. Evaporation of the solvent left 22.7 mg of crude metallated product. Column chromatography provided 11.0 mg of pure product which was free from impurities due to possible contamination of the 1-(1-hydroxyethyl)-uracyl with 2-5% of 3-(1-hydroxyethyl)-uracyl and/or 1,3-bis-(1-hydroxyethyl) uracyl.

R_f = 0.15 (on SiO_2 eluted with CH_2Cl_2 : MeOH = 98 : 2).

^{1}H-NMR (200 MHz, $CDCl_3$) : 9.38 (6H, sharp multiplet, 2,- 8-, 12-, 13-, 17-, 18-H) 8.74 (2H, d, J = 4.9 Hz, 3-, 7-H), 8.30 (2H, d, J = 7.9 Hz, 3'-, 5'-H), 8.23 (2H, d, J = 8.5 Hz, 2'-, 6'-H), 8.07 (1H, broad, 3"-NH), 7.26 (1H, d, J = 7.9 Hz, partially obscured by the solvent peak, 6"-H), 5.68 (1H, d, J = 7.9 Hz, 5"-H), 4.96 (6H, m, 1^{IV}- CH_2), 4.60 (2H, broad t, OCH_2), 4.10 (2H, broad t, NCH_2), 2.47 (6H, quintet, 2^{IV}- CH_2), 1.82 (6H, quintet, 3^{IV}-CH_2), 1.53 (6H, m, 4^{IV}-CH_2), 1.26 (36H, s, 5^{IV} to10^{IV}- CH_2), 0.88 (9H, t, 11^{IV}-CH_3).

^{13}C-NMR (100 MHz, $CDCl_3$): 166.43 (COO), 162.92 (4"), 150.35, 149.70 (2"), 149.64, 149.04 and 148.59 (C_α-pyrrole), 144.67 (6"), 134.68 (2'-, 6'-C), 131.20, 129.12, 128.99 and 128.85, (C_β-pyrrole) 128.02 (4'-C), 127.70 (3'-, 5'-C), 120.81 (15- C), 120.35 (10, 20-C), 116.93 (5-C), 102.33 (5"-C), 62.31 (OCH_2), 48.10 (NCH_2), 39.05 (2^{IV}-CH_2), 35.69 (1^{IV}-CH_2), 31.95 (9^{IV}-CH_2), 30.79 (3^{IV}-CH_2), 29.79, 29.70 and 29.40 (4^{IV} to 8^{IV}-CH_2), 22.73 (10^{IV}-CH_2), 14.15 (11^{IV}-CH_3).

FAB-MS: 1092.6 (M)+, 951.4 (M-$C_{10}H_{21}$)+.

Esterification with porphyrinic free acid B to B-P

Acid B-COOH (73.3 mg, 0.082 mmoles), DMAP (16.4 mg, 0.13 mmoles), EDCI (28.8 mg, 0.15 mmoles) and 2,6-diacetamido-4-(2-hydroxyethyloxy)-pyridine (33.7 mg, 0.13 mmoles) were stirred in dry dichloromethane (40 mL) overnight under argon atmosphere. Dilution with dichloromethane (40 mL) and washing twice with saturated aqueous ammonium chloride then twice with brine, drying over anhydrous sodium sulphate and evaporation of the solvent left a residue which was chromatographed on silica gel (ϕ = 4 cm, H = 8 cm). The main band (third fraction) left after evaporation of the solvent 77.2 mg of pure product (yield = 83.5 %). The fifth fraction (5.5 mg) is the acylurea B-AU.

R_f = 0.23 (SiO_2 eluted with CH_2Cl_2)

^{1}H-NMR (200 MHz, $CDCl_3$) : 9.50 (4H, sharp quartet J = 4.9 Hz, 12-, 13-, 17-, 18- H), 9.38 (2H, d, J = 4.9 Hz, 2-, 8-H), 8.75 (2H, d, J = 4.9 Hz, 3-, 7-H), 8.46 (2H, d, J = 7.9 Hz, 3'-, 5'-H), 8.25 (2H, d, J = 8.5 Hz, 2'-, 6'-H), 7.69 (2H, s, 3"-, 5"-H), 7.61 (2H, broad s, NH), 4.93 and 4.90 (8H, m, 1^{IV}-CH_2 and $COOCH_2$), 4.57 (2H, broad t, OCH_2), 2.51 (6H, quintet, 2^{IV}-CH_2), 2.19 (6H, s, $COCH_3$), 1.79 (6H, quintet, 3^{IV}-CH_2), 1.52 (6H, m, 4^{IV}-CH_2), 1.28 (36H, s, 5^{IV} to10^{IV}-CH_2), 0.88 (9H, t, 11^{IV}-CH_3), -2.66 (2H, s, NH).

^{13}C-NMR (100 MHz, $CDCl_3$): 168.53 (CONH), 166.78 (COO), 150.65 (2"-, 6"-C) 147.87 (4"-C), ~146.5 (very broad C_α-pyrrole), 134.51 (2'-, 6'-C), 128.99 (4'-C), ~128.5 (broad, C_β-pyrrole), 128.08 (3'-, 5'-C), 119.92 (15-C), 119.44 (10, 20-C), 116.46 (5-C), 96.33 (3", 5"-C), 66.49 ($COOCH_2$), 63.15 (OCH_2), 39.00 and 38.79 (2^{IV}-CH_2), 35.89 and 35.49 (1^{IV}-CH_2), 31.96 (9^{IV}-CH_2), 30.72 and 30.64 (3^{IV}-CH_2), 29.76, 29.68 and 29.39 (4^{IV} to 8^{IV}-CH_2), 24.87 ($COCH_3$), 22.73 (10^{IV}-CH_2), 14.16 (11^{IV}-CH_3). N.B. the 1'-C is probably overlapping.

FAB-MS: 11128.6 (M+H)+, 986.5 (M+H-C10H22)+, 894.5 (benzylic cleavage of the pyridine moiety), 847.5 (M+H-$C_{10}H_{22}$ -$C_{10}H_{20}$).

Esterification with porphyrinic free acid C to U-C-U

Diacid C-$(COOH)_2$ (155.3 mg, 0.18 mmoles) was sonicated in 70 mL dry dichloromethane to suspend evenly and dissolve slowly as the reaction proceeds. DMAP (48.6 mg, 0.4 mmoles), EDCI (75.9 mg, 0.4 mmoles) and 1-(2-hydroxyethyl)-uracyl (84.31 mg, 0.54 mmoles) were strired in the dark at room temperature over night. Aqueous work-up (NH_4Cl, NaCl), and drying (Na_2SO_4) gave 98.1 mg of crude reaction mixture which was chromatographed on silica gel (80 mL, Φ = 2.5 cm, H = 22 cm), eluted with CH_2Cl_2 : MeOH, 95 : 5. The first main fraction is U-C-U which still contains about 15 % the 3-hydroxyethyluracyl derivative (17.5 mg). One recrystallization from AcOEt gives a dark powder with about 5 % of the other uracyl derivative. After three recrystallizations a pure sample could be obtained as inferred from the ^{1}H-NMR spectrum.

R_f = 0.80 (SiO2 eluted with CH_2Cl_2 : MeOH, 3 : 1)

^{1}H-NMR (200 MHz, $CDCl_3$): 9.44 (4H, d, J = 4.9 Hz, 2-, 8-, 12-, 18-H), 8.78 (4H, d, J = 4.9 Hz, 3-, 7-,13-, 17-H), 8.39 (4H, d, J = 7.9 Hz, 3'- and 5'-H), 8.26 (4H, d, J = 8.5 Hz, 2'- and 6'-H), 7.39 (2H, d, J = 7.9 Hz, 6"-H), 5.82 (2H, d, J = 7.9 Hz, 5"-H), 4.95 (4H, t, 1"-CH_2), 4.79 (4H, broad t, J ~ 5 Hz, OCH_2), 4.28 (4H, broad t, J ~ 5 Hz, N-CH_2), 2.50 (4H, broad quintet, 2"-CH_2), 1.77 (4H, broad quintet, 3"-CH_2), 1.55(4H, m, 4"-CH_2), 1.24 (24H, s, 5"-10"-CH_2), 0.85 (6H, t, 11"-CH_3), -2.70 (2H, s, NH).

^{13}C-NMR (100 MHz, $CDCl_3$): 166.37 (COO), 162.96 (4"-C), 150.52 (2"-C), 148.27 (1'-C), 144.75 (6"-C), 134.71 (2'-, 6'-C), ~131.0 (broad, 3-, 7-, 13-, 17-C), 128.58 (4'- C), 127.86 (3'-, 5'-C), 120.57 (10-, 20-C), 117.46 (5-, 15-C), 102.49 (5"-C), 62.54 (OCH_2), 48.24 (NCH_2), 38.85 (2"-CH_2), 35.40 (1"-CH_2), 31.91 (9"-CH_2), 30.59 (3"- CH_2), 29.70, 29.63 and 29.34 (4"-8"-CH_2), 22.69 (10"-CH_2), 14.12 (11"-CH_3).

FAB-MS: 1135.7 (M+H)+, 1024.6 (cleavage of the uracyl moiety), 952.6 (cleavage of $COOCH_2CH_2$-Uracyl).

U-C(Zn)-U

Zinc metallation of **U-C-U** with zinc acetate in a mixture of methanol and dichloromethane followed by stirring at room temperature for 90 min leaves a crude reaction mixture which still had some unmetallated porphyrin. Careful chromatography on silica gel separates neatly the unmetallated product which elutes just before the desired zinc porphyrin. Monitoring can be accomplished by Vis absorption spectroscopy. It is not very soluble in chloroform.

R_f = 0.82 (SiO_2 eluted with $CH2Cl_2$: MeOH, 4 : 1)

^{1}H-NMR (200 MHz, $CDCl_3$): 9.57 (4H, d, J = 4.9 Hz, 2-, 8-, 12-, 18-H), 8.89 (4H, d, J = 4.9 Hz, 3-, 7-,13-, 17-H), 8.37 (4H, d, J ~ 8 Hz, 3'- and 5'-H), 8.30 (4H, d, J = 8.5 Hz, 2'- and 6'-H4), 8.10 (2H, broad s, NH), 7.37 (2H, d, J = 7.9 Hz, 6"-H), 5.79 (2H, d, J = 7.3 Hz, 5"-H), 5.00 (4H, broad t, 1"-CH_2), 4.79 (4H, t, OCH_2), 4.28 (4H, t, N-CH_2), 2.56 (4H, broad quintet, 2"-CH_2), 1.83 (4H, broad quintet, 3"-CH_2), 1.55 (4H, m, 4"-CH_2), 1.25 (24H, s, 5"-10"-CH_2), 0.87 (6H, t, 11"-CH_3).

FAB-MS: 1197.4 (M+H)+ . In the crude mixture a peak was present at 1241.5 arising from the dihydroxyethyl-uracyl impurity. This peak was absent in the purified product.

Esterification with porphyrinic free acid C to P-C-P

Diacid C (178.7 mg, 0.21 mmoles) was sonicated in dry dichloromethane. DMAP (25.6 mg, 0.21 mmoles), EDCI (80.30 mg, 0.42 mmoles) and 2,6- diacetamido-4-(2-hydroxyethyloxy)-pyridine (159.1 mg, 0.628 mmoles) were added and the mixture was stirred under argon, at room temperature, in the dark, overnight. Aqueous work-up (NH_4Cl, NaCl) and drying (Na_2SO_4) left a residue which was chromatographed on silica gel by elution with 3% methanol in dichloromethane. The major fourth band corresponded to pure P-C-P which was recrystallized from boiling ethyl acetate to which *n*-hexane was added until the mixture slightly clouded. After storing in the refrigerator (-18 °C) the filtered dry powder weighed 86.5 mg corresponding to a yield of 31 %.

R_f = 0.20 (SiO_2 eluted with CH_2Cl_2 : MeOH, 97 : 3)

^{1}H-NMR (200 MHz, $CDCl_3$): 9.43 (4H, d, J = 4.9 Hz, 2-, 8-, 12-, 18-H), 8.81 (4H, d, J = 4.9 Hz, 3-, 7-,13-, 17-H), 8.46 (4H, d, J = 7.9 Hz, 3'- and 5'-H), 8.27 (4H, d, J = 8.5 Hz, 2'- and 6'-H), 7.68 (4H, s, 3"- and 5"-H), 7.57 (2H, s, NH), 4.95 and 4.87 (8H, m, 1"-CH_2 and $COOCH_2$), 4.57 (4H, broad t, OCH_2), 2.50 (4H, broad quintet, 2"-CH_2), 2.20 (12H, s, $COCH_3$), 1.78 (4H, broad quintet, 3"-CH_2), 1.55 (4H, m, 4"- CH2), 1.26 (24H, s, 5"-10"-CH_2), 0.85 (6H, t, 11"-CH_3), -2.70 (2H, s, NH).

^{13}C-NMR (100 MHz, $CDCl_3$): 168.55 (CONH), 166.71 (COO), 150.64 (2"-, 6"-C) 147.80 (4"-C), ~146.5 (very broad C_α-pyrrole), 134.49 (2'-, 6'-C), 131.47 (broad, C_β-pyrrole), 129.14 (4'-C), 128.05 (3'-, 5'-C), 120.37 (10, 20-C), 117.74 (5, 15-C), 96.31 (3", 5"-C), 66.47 ($COOCH_2$), 63.16 (OCH_2), 38.82 (2^{IV}-CH_2), 35.39 (1^{IV}-CH_2), 31.91 (9^{IV}-CH_2), 30.60 (3^{IV}-CH_2), 29.72, 29.64 and 29.34 (4^{IV} to 8^{IV}-CH_2), 24.89 ($COCH_3$), 22.69 (10^{IV}-CH_2), 14.12 (11^{IV}-CH_3). N.B. the 1'-C is probably overlapping.

FAB-MS: 1329.8 (M+H)+, 1187.6 (M+H - $C_{10}H_{22}$).

P-C(Zn)-P

Was prepared in pure form on a 30 mg scale as described above for **U-(Zn)-U** in nearly quantitative yield. Attempts to recrystallize it from hot AcOEt/*n*-hexane failed, but the cloudy colloidal suspension in dilute dry *n*-hexane could be centrifuged (Hettich Universal 16 centrifuge equipped with a 1624 rotor, 4000 rpm, 5 min at room temperature), dried in vacuum and collected.

Details of the porphyrinic acylurea which is sometimes obtained as a by-product in EDCI couplings can be obtained from Silviu Balaban.