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Mono-amine Functionalized Phthalocyanines: Microwave-Assisted Solid-Phase Synthesis and Bioconjugation Strategies

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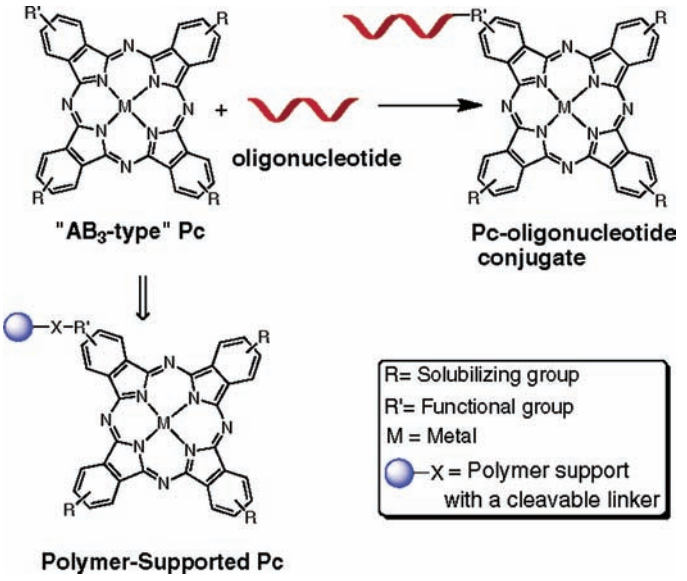

Phthalocyanines (Pcs) are excellent candidates for use as fluors for near-infrared (near-IR) fluorescent tagging of biomolecules for a wide variety of bioanalytical applications. Monofunctionalized Pcs, having two different types of peripheral substitutents, one for covalent conjugation of the Pc to biomolecules and others to improve the solubility of the macrocycle, are ideally suited for the desired applications. To date, difficulties faced during the purification of monofunctionalized Pcs limited their usage in various types of applications. Herein are reported a new synthetic method for rapid synthesis of the target Pcs and bioconjugation techniques for labeling of the oligonucleotides with the near-IR fluors. A novel synthetic route was developed utilizing a hydrophilic, poly(ethylene glycol) (PEG)-based support with an acid-labile Rink Amide linker. The Pcs were functionalized with an amine group for covalent conjugation purposes and were decorated with short PEG chains, serving as solubilizing groups. Microwave-assisted solid-phase synthetic method was successfully applied to obtain pure asymmetrically substituted monoamine functionalized Pcs in a short period of time. Three different bioconjugation techniques, reductive amination, amidation, and Huisgen cycloaddition, were employed for covalent conjugation of Pcs to oligonucleotides. The described microwave-assisted bioconjugation methods give an opportunity to synthesize and isolate the Pc-oligonucleotide conjugate in a few hours.

Introduction

Fluorescence-based detection is the mainstay of many bioassays including immunoassays, flow cytometry, DNA sequencing, nucleic acid diagnostics, proteomics, and other clinical chemistry applications.[1,2] Increasing requirements for sensitivity and decreased scale as in capillary and

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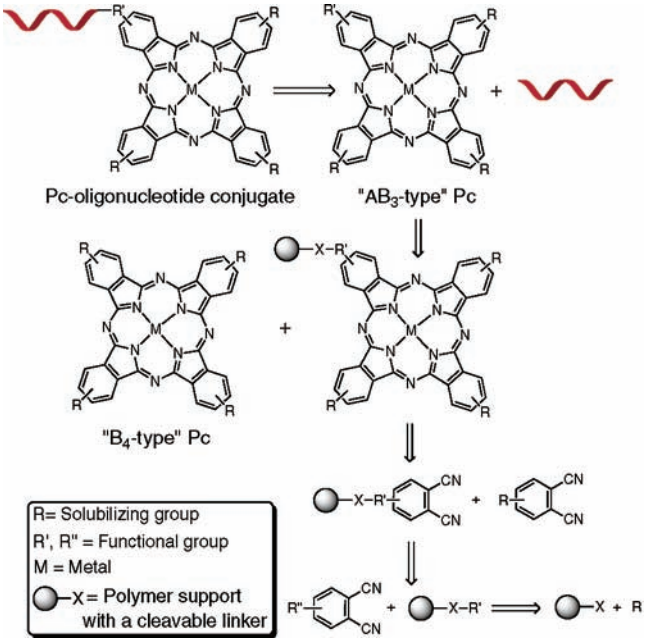
microchip devices has put increasing demands on the efficiency of these assays. One method to improve fluorescence-based detection is the use of near-IR dyes, which have decreased background due to scattering and native fluorescence in this region of the spectrum (650−850 nm).[3] Our laboratory and a number of others have been developing phthalocyanines (Pcs) as near-IR fluors for bioanalytical applications. Pcs have a number of improved properties such as narrow absorbance and emission spectra, resistance to photobleaching, and tunability of spectral and photophysical properties by variation of substituents around the Pc aromatic core or the central metal.[4] Applications of Pcs in bioassays included analysis of PCR products,[5] single gene mutation detection[6] and resonance energy transfer-based assays.[7] Structural requirements of Pcs for most bioanalytical applications include the need for water-solubility and a unique functional group for attachment to the biomolecule. Asymmetrically substituted Pcs with two different types of peripheral substituents, one reactive group for conjugation and others for solubility, are ideally suited for these applications, but difficulties in the isolation of these Pcs have limited their use in biological and bioanalytical applications. Recently, we have reported solid-phase synthesis of mono-hydroxylated Pcs.[8] Herein we present a time-efficient, microwave-assisted method for synthesis of pure "AB_3-type" asymmetrically substituted, monoamine-functionalized Pcs on solid support along with methods for their ready conjugation to oligonucleotides.

Results and Discussion

Generalized retrosynthetic analysis of a functionalized Pc-biomolecular conjugate is shown in Scheme 1. The stepwise synthesis starts with immobilization of one phthalonitrile to the polymer support via a cleavable linker. Cyclotetramerization of the polymer-bound phthalonitrile with the second type of phthalonitrile in solution yields the "AB_3-type" target Pc on the polymer support. Following the cleavage of the desired mono-functionalized Pc from the polymer support, in the final step, the AB_3-type Pc is covalently conjugated to a biomolecule by a variety of conjugation strategies.

Scheme 2 shows the synthesis of an amine-functionalized, solid-supported phthalonitrile as the starting point for Pc synthesis. Poly(ethylene glycol) (PEG)-based Rink Amide ChemMatrix resin with initial loading of 0.52 mmol/g was employed as the polymer support. Synthesis of the monoamine functional Pcs **7b** and **8b** started with incorporation of the amine functionality to the solid support via coupling of 0.1 mmol Fmoc-Lys(Mtt)-OH according to standard protocols.[9] The ε-amine of the lysine will provide the functional handle for bioconjugation

SCHEME 1. Generalized Retrosynthetic Analysis of Mono-functionalized Pc-Biomolecular Conjugates



SCHEME 2. Immobilization of the Amino Acid and Phthalonitrile to the Polymer Support

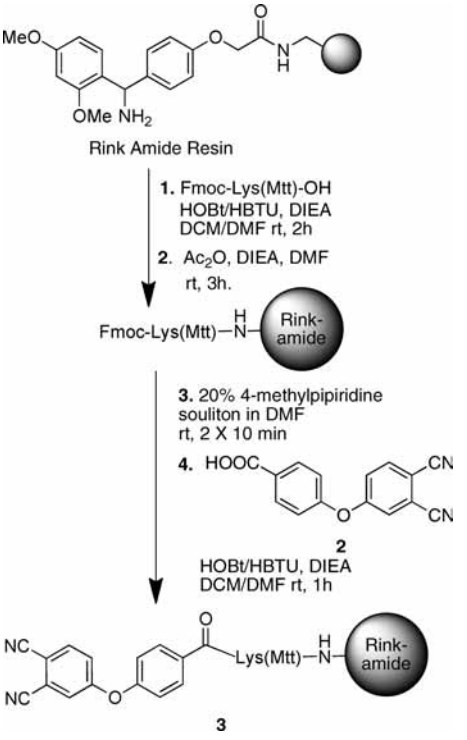


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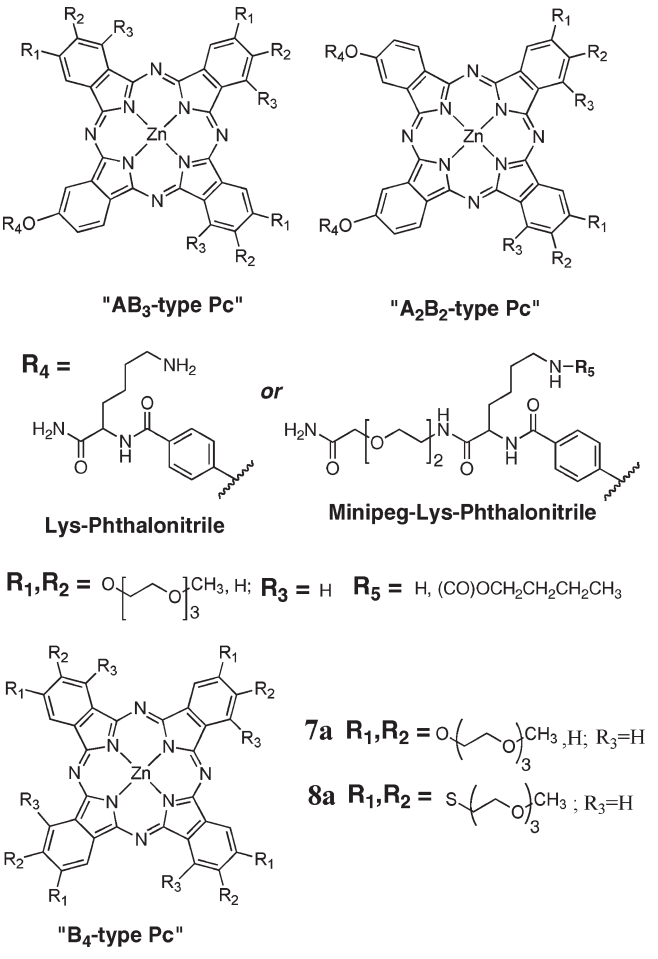
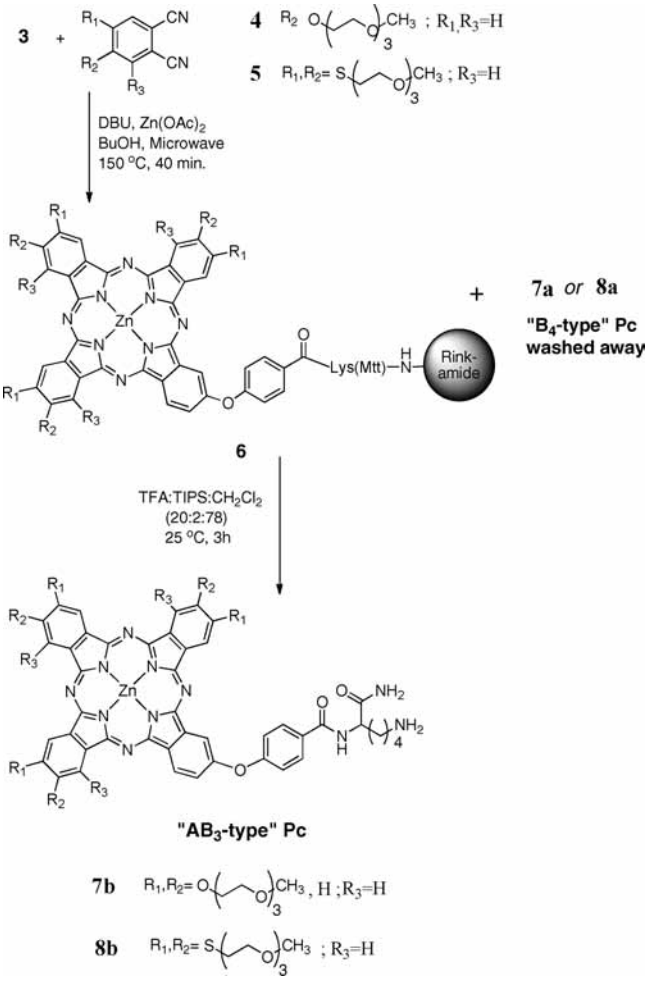
TABLE 1. Optimization of the Cyclotetramerization Reaction

	sequence on the resin	phthalonitrile loading on resin (mmol/g)	reaction concn (M)/equiv of phthalonitrile in solution relative to solid support	reaction conditions	solvent	outcome of the reaction/crude mixture yield (%)
1	Minipeg-Lys(Boc)-phthalonitrile	0.46	0.16/9	microwave, 30 min, 200 °C	BuOH	mixture of all Pc congeners/19
2	Minipeg-Lys(Boc)-phthalonitrile	0.23	0.16/9	microwave, 30 min, 180 °C	BuOH	mixture of AB_3, A_2B_2 and (AB_3+100)/21
3	Minipeg-Lys(Mtt)-phthalonitrile	0.10	0.2/12	microwave, 40 min, 150 °C	dodecanol	AB_3:A_2B_2 (2:1)/22
4	Lys(Mtt)-phthalonitrile	0.30	0.2/12	microwave, 40 min, 150 °C	dodecanol	AB_3:A_2B_2 (1:1)/18
5	Lys(Mtt)-phthalonitrile	0.10	0.3/15	microwave, 40 min, 150 °C	dodecanol	AB_3:A_2B_2 (97:3)/22
6	**Lys(Mtt)-phthalonitrile**	**0.10**	**0.8/24**	**microwave, 40 min, 150 °C**	**BuOH**	**AB_3 > 99%/15−27**
7	Lys(Mtt)-phthalonitrile	0.10	0.8/24	thermal heat, 24 h, 120 °C	BuOH	AB_3 > 99%/7

SCHEME 3. Structures of Most Common Pc Congeners Formed during the Cyclotetramerization Reaction



SCHEME 4. Solid-Phase Synthesis of AB_3-Type Pcs



after removal of the acid-labile 4-methyltrityl (Mtt) protecting group during the cleavage of the Pc from the polymer support. The remaining amine sites of the resin **1** were capped with an excess of Ac_2O. Following the removal of the 9-fluorenylmethoxycarbonyl (Fmoc) protecting group, carboxylate phthalonitrile **2** was anchored to the amino terminus of the polymer-bound lysine to give a polymer-supported phthalonitrile **3** with 0.1 mmol/g loading (Scheme 2).

As a result of the dynamic nature of the polymer support, the outcomes of the solid-phase reactions are affected by various factors such as the reaction time and temperature, loading of the resin, linker length, solvent, and the concentration of the reaction.[10] Base-promoted phthalonitrile

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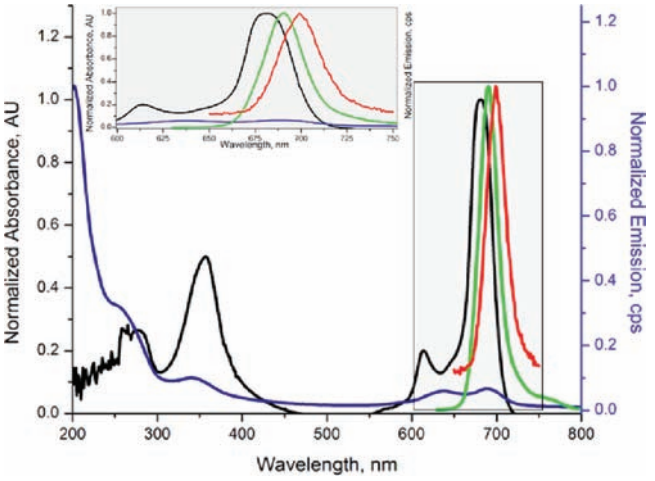


FIGURE 1. Absorbance-emission spectra of ZnPc **7b** and bioconjugate **9**: black, ZnPc **7b** $\lambda max_{(abs)}$ = 681 nm (DMSO); green, ZnPc **7b** $\lambda max_{(em)}$ = 691 nm (DMSO); blue, bioconjugate **9** $\lambda max_{(abs)}$ = 640, 690 nm (H_2O); red, bioconjugate **9** $\lambda max_{(em)}$ = 699 nm (pH 8.5 buffer).

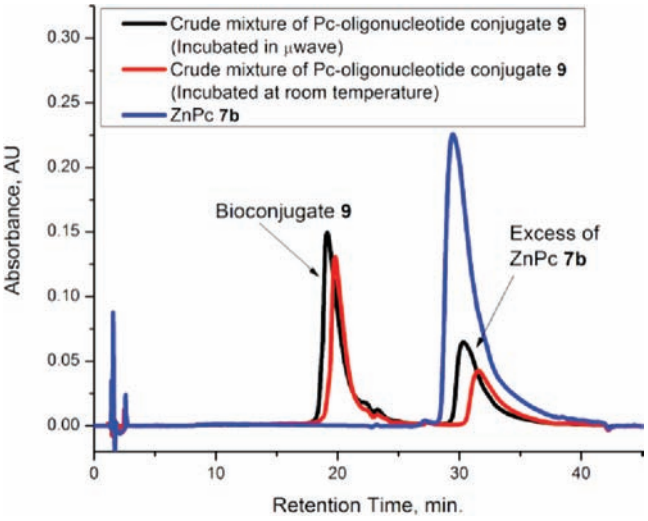
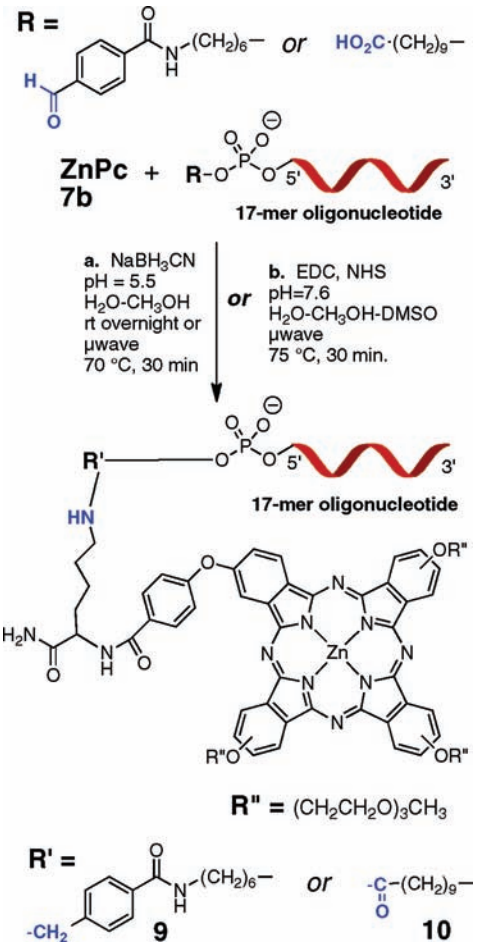


FIGURE 2. Labeling of the oligonucleotide with ZnPc **7b** by reductive amination. The chromatograms absorbance data were extracted at λ = 680 nm.

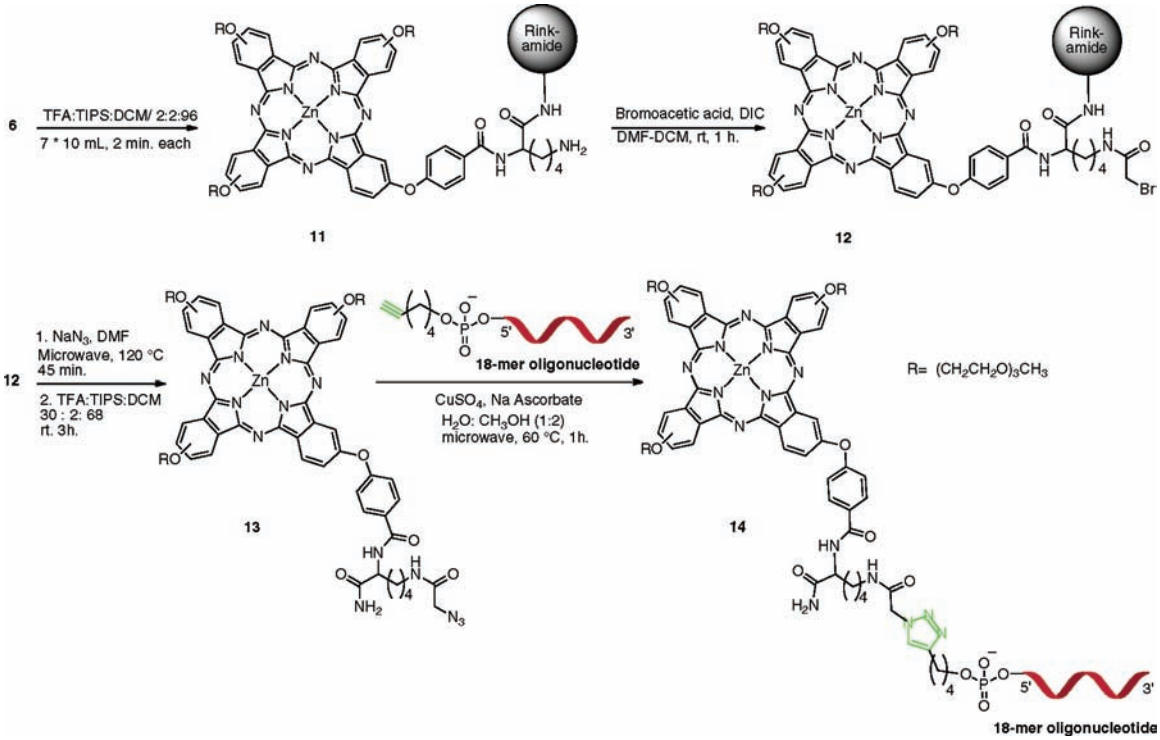
tetramerization reaction was optimized on the basis of these criteria as summarized in Table 1. Experiments were performed in CEM MARS extraction system using fiber optic temperature and pressure probes. Early experiments showed that higher phthalonitrile loadings (0.46 mmol/g), determined on the basis of the absorption of the Fmoc adduct, and longer linker lengths lead to the formation of other Pc congeners (A_2B_2, A_3B, etc.) on the resin via site−site interactions (Table 1) (Scheme 3). The affect of the linker length on the outcome of the cyclotetramerization reaction was examined by incorporating commercially available Fmoc-8-amino-3,6-dioxaoctanoic acid (Fmoc-miniPEG-(OH)) as a spacer (Table 1, entries1−3) (Scheme 3). As a result of an increased mobility of the reactive sites, it was not possible to have complete site isolation even at lower loadings (0.1 mmol/g). Thus, the spacer was excluded from the sequence. Solvent effect is another factor regulating site interactions on the polymer support.[10] Solvents in which the resin swelling is minimal promote site isolation by limiting the mobility of the reactive sites on the resin.[10] Thus, the cyclotetramerization reaction was performed in dodecanol. However, the selected solvent did not have a significant effect on the outcome of the reaction. Although neither the reaction time nor the temperature affected the outcome of the cyclotetramerization reaction, at elevated reaction temperatures (≥180 °C) we saw the decomposition of the PEG resin. Thus, the following experiments were performed at 150 °C. The protecting group on the ε-amine of the lysine has a critical effect on the outcome of the cyclotetramerization reaction. Initially, we employed lysine with a *tert*-butyloxycarbonyl (Boc) protecting group on the ε-amine, and MALDI-MS mass spectrometry analysis of the cleaved product showed a major ion at *m*/*z* M+100 (Table 1, entry 2) (Scheme 3), corresponding to displacement of the *tert*-butyl group of the Boc protecting group with a *n*-butyl group of the solvent, butanol (Scheme 3). Further proof for this transition was obtained by performing the reaction in different alcohols, such as *n*-pentanol and 2-butanol (data not shown). Because of the inseparable mixtures obtained with Boc-protected amine, Fmoc-Lys(Mtt)-OH was employed

SCHEME 5. Conjugation of ZnPc 7b to an Oligonucleotide by Reductive Amination or Amidation



for the later experiments. The last parameter that we evaluated to optimize the base-promoted cyclotetramerization reaction was the reaction concentration. Since at high reaction concentrations molecules in solution occupy most of the

SCHEME 6. Synthesis of Pc-Oligonucleotide Conjugate 14 via Huisgen Cycloaddition



reactive sites,[10] high degree of site isolation is achieved. We successfully suppressed the formation of A_2B_2-type Pc by increasing the phthalonitrile concentration in solution from 0.3 to 0.8 M (Table 1, entries 5 and 6). On the basis of these results, optimized conditions for the base-promoted cyclotetramerization reaction were determined as shown in Table 1, entry 6.

A suspension of polymer-bound phthalonitrile **3**, tri(ethylene glycol)-substituted phthalonitrile **4**,[8] $Zn(OAc)_2$, and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in BuOH were irradiated for 40 min in a microwave to give polymer-bound AB_3-type ZnPc **6** and the corresponding symmetrically substituted B_4-type Pc **7a** in solution (Scheme 4). ZnPc **7a**[8] was removed from the mixture by washing the resin with hot BuOH. Following the cleavage of the target Pc from the polymer support, asymmetrically substituted ZnPc **7b** was simply purified by filtration through LH-20 column and was obtained in 27% yield without any contamination with other Pc congeners. Water-soluble oligo(ethylene glycol)-subsituted ZnPc **7b** exhibits sharp fluorescence emission in the near-IR region of the electromagnetic spectrum, which is highly favorable for the desired bioanalytical applications (Figure 1).

The same method was employed to synthesize ZnPc **8b,** decorated with six tri(ethylene glycol) chains. Synthesis of ZnPc **8b** endorsed the applicability of the method to different types of phthalonitriles (Scheme 4).

The successful synthesis of the monoamine-functionalized oligo(ethylene glycol)-substituted AB_3-type Pcs was followed by the covalent labeling of oligonucleotides with ZnPc **7b** utilizing three different bioconjugation techniques. All Pc-oligonucleotide conjugates were successfully isolated by reverse-phase ion-pair HPLC. If desired, excess of the Pcs can be recycled via simple purification to be used for the next reaction. Initially, ZnPc **7b** was conjugated to two different 17-mer oligonucleotides, one of which was functionalized with a carboxylic acid via a C_{10}-linker at the 5′-end and the other of which was modified with a 4-formyl *N*-hexylbenzamide linker at the 5′-end (Scheme 5). While bioconjugate **9** was synthesized via reductive amination by incubating the reaction in a microwave at 70 °C for 30 min. (79% labeling efficiency calculated based on the absorbance of the unreacted oligonuclotide), as well as at room temperature overnight (Scheme 5, Figure 2), synthesis of bioconjugate **10** was achieved by amidation in a microwave at 75 °C for 30 min (35%) (Scheme 5). As a result of the competition between the hydrolysis of the ester, formed in situ, and amide bond formation, the bioconjugate **10** was obtained in low yield relative to bioconjugate **9**. Comparison of the two conjugation techniques revealed that the synthesis of Pc-oligonucleotide conjugates could be achieved in relatively high yields by employing a reductive amination route. From the results above it has been concluded that while microwave irradiation did not significantly affect the labeling efficiency of the oligonucleotide (Figure 2), it reduced the reaction time from hours to minutes. Thus, the following experiments were performed in a microwave.

Figure 1 shows the absorbance-emission spectra of the bioconjugate **9** in aqueous medium. Bioconjugate **9** was aggregated in water as judged by the split Q-band in the absorbance spectrum. However, it had a sharp emission band at 699 nm, with 9 nm of Stokes shift, in pH 8.5 buffer.

The scope of the Pc-oligonucleotide bioconjugation techniques was extended with a Huisgen cycloaddition, so-called "click chemistry".[11] For the ease of the purification as well as

(11) Vsevolod, V. R.; Green, L. G.; Fokin, V. V.; Sharpless, K.B. A A stepwise Huisgen cycloaddition process: Copper(I)-catalyzed regioselective ligation of azides and terminal alkynes. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599.

to reduce the reaction time, the reactions were performed on the solid support under microwave irradiation (Scheme 6). In order to remove the Mtt protecting group without cleaving the Pc from the solid support, polymer-supported ZnPc **6** was treated with 2% trifluoroacetic acid (TFA) solution to yield resin-bound ZnPc **11** (Scheme 6). Bromoacetylation of the ε-amine of the polymer-bound ZnPc **11** to yield ZnPc **12** was followed by treating the resin with NaN_3 in a microwave for 45 min to give the azide-functionalized resin-bound ZnPc **13** (Scheme 6).[12] Azide Pc **13** was cleaved from the solid support, purified by running through a LH-20 column, and obtained in 19% overall yield. Conjugation of ZnPc **13** to an 18-mer oligonucleotide, bearing a hexynyl linker at the 5′-end, was carried in a microwave at 60 °C for 1 h (Scheme 6).[13] Pc-oligonucleotide bioconjugate **14** was obtained with 80% labeling efficiency, which was calculated on the basis of the absorbance of the unreacted oligonucleotide.

Conclusion

The presented solid-phase synthetic method is an imperative approach to synthesize monoamine-functionalized water-soluble Pcs in a short period of time. Coupling of the effective method with microwave irradiation not only improved the yield of the cyclotetramerization reaction but also reduced the reaction time from hours to minutes.[8] Covalent conjugation of Pcs to oligonucleotides, modified with various types of functional groups, was successfully achieved by employing different types of bioconjugation techniques. The oligonucleotides were efficiently labeled and isolated in high yields. The discussed synthetic methods are being applied to synthesis of a wide variety of Pcs and Pc-oligonucleotide conjugates for bioanalytical applications.

Experimental Section

Polymer-Supported Fmoc-Lys-(Mtt)-OH 1. Prior to the synthesis, the Rink Amide resin (0.4 g) with 0.52 mmol/g loading capacity was swelled in CH_2Cl_2 (20 mL) for 30 min. The solvent was filtered off, and CH_2Cl_2 (7 mL) was added to the well-swelled resin. Fmoc-Lys-(Mtt)-OH (0.05 mmol, 31 mg), 1-hydroxybenzotriazole (HOBt) (0.05 mmol, 6.7 mg), and *O*-(1-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (0.05 mmol, 19 mg) were dissolved in *N*,*N*-dimethylformamide (DMF) (1 mL). The solution was added to the resin in CH_2Cl_2. Diisopropylethyl amine (DIEA) (0.1 mmol, 17.4 μL) was added to the suspension, and the reaction flask was placed in a shaker and kept at room temperature for 1 h to give the resin **1** with 0.1 mmol/g of loading. The resin was washed with CH_2Cl_2 (4 × 15 mL), DMF (2 × 15 mL), and CH_2Cl_2 (4 × 15 mL).

Acetylation of the Polymer Support. The resin **1** was suspended in 0.28 M solution of DIEA in DMF (25 mL). Ac_2O (8.4 mmol, 0.8 mL) was added to the suspension, and the reaction was placed in a shaker and kept at room temperature for 3 h. The resin was washed with DMF (5 × 15 mL) and CH_2Cl_2 (5 × 15 mL).

General Procedure for Fmoc Cleavage. The resin was suspended in 20% (v/v) solution of 4-methyl piperidine in DMF (10 mL). The reaction was placed in a shaker and kept at room temperature for 10 min. Following the filtration of the solution, fresh cleavage cocktail (10 mL) was added to the resin, and the reaction was shaken 10 more min at room temperature. The resin was washed with DMF (5 × 15 mL), CH_2Cl_2 (3 × 15 mL) and next was submitted to amino acid (phthalonitrile) coupling.

Determination of Loading Capacity of the Phthalonitrile-Bound Resin. Before the acetylation step, ∼50 mg of the polymer-supported Fmoc-Lys-(Mtt)-OH (**1**) was placed in 5 mL syringe equipped with coarse frit and dried in the desiccator for 4−5 h; 6−7 mg of the dry resin was placed in a 3 mL syringe equipped with coarse frit and swelled in CH_2Cl_2 (1 mL) for 30 min. Following the filtration of the CH_2Cl_2, the resin was placed in 20% (v/v) 4-methyl piperidine solution in DMF (1 mL) and kept in the cleavage solution for 20 min. The solution was collected by filtration, and the resin was washed with CH_3OH (3 × 2 mL). Combined filtrate solution (7 mL) was detected with electronic absorption spectrometry. The loading capacity (mmol/g) of the resin was calculated using the following equation:

$$(UV_{300} \times 10^6 \times 0.007)/(7800 \times \text{mg of resin})$$

Polymer-Supported 4-(3,4-Dicyanophenoxy)Benzoic Acid 3. The resin **1** (0.4 g) with 0.1 mmol/g Lys-(Mtt)-OH loading was placed in CH_2Cl_2 (3 mL). HOBt (0.44 mmol, 59 mg), HBTU (0.44 mmol, 167 mg) and the carboxylated phthalonitrile **2** (0.44 mmol, 110 mg) were dissolved in DMF (1 mL). The solution and DIEA (0.88 mmol, 0.15 mL) were added to the resin, and the reaction flask was placed in a shaker and kept at room temperature for 2 h. (In order to have an efficient coupling yield, amino acid and/or carboxylated phthalonitrile concentration in the reaction was adjusted to 0.2 M.) The resin was washed with CH_2Cl_2 (4 × 15 mL), DMF (3 × 15 mL), and CH_2Cl_2 (3 × 15 mL). FT-IR (KBr, cm^{-1}): 3515, 2863, 2232, 1751, 1738, 1656, 1610, 1588, 1542, 1508, 1452, 1348, 1300, 1177, 1146, 1088, 1031, 991, 951, 937, 920, 864, 840, 801, 773, 751, 709, 660, 639.

General Procedure for Synthesis of AB_3-Type Pcs 7b, 8b, and 13. The resin **3** (0.4 g) was swelled in BuOH (2 mL) for 30 min in the microwave vessel. Corresponding phthalonitrile (5 mmol) and $Zn(OAc)_2$ (1.4 mmol, 0.25 g) were placed in a 20 mL glass vial in BuOH (4 mL) and heated up to 90 °C in water bath. DBU (2.8 mmol, 0.38 mL) was added to the suspension, and the mixture was vigorously stirred until all of the metal salt was dissolved. The solution was quickly transferred to the microwave vessel, having the resin **3** with BuOH (2 mL). The reaction was irradiated in the microwave at 850 W. The temperature was ramped to 150 °C in 5 min and was held at the temperature for 40 min. The resin was washed, until a colorless filtrate was obtained, first with hot BuOH (12 × 25 mL) and then hot CH_2Cl_2 (3 × 25 mL).

General Procedure for Cleavage of AB_3-Type Pc 7b, 8b, and 13. The resin was suspended into a solution of TFA/CH_2Cl_2/TIPS (triisopropylsilane) (20:78:2; 25 mL) (TFA concentration was increased to 30% for the cleavage of the ZnPc **13**) and agitated in the shaker for 3 h at room temperature. Filtrate was evaporated to dryness, and the crude mixture was purified by filtration through a LH-20 column using CH_3OH as eluent (see Supporting Information for ZnPc **8b**).

ZnPc 7b. Obtained as a blue solid (14 mg, 27% yield). Condition B was employed to perform HPLC. t_R = 29.45 min. MALDI-MS: calcd $C_{66}H_{75}N_{11}O_{15}Zn^+$ 1325.47; found 1324.98. λ_{max}(abs) (DMSO): 681 ± 1 nm (log ε = 5.2). λ_{max} (em) (DMSO): 691 nm. ϕ_f: 0.10.

General Procedure for Purification of Symmetrically Substituted Pcs 7a and 8a. Filtrate solutions were combined and evaporated to dryness. The crude mixture was dissolved in a minimum amount of CH_3OH, and a large excess of diethyl ether

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(30X) was added to solution. The solution was kept at −20 °C overnight, and the suspension was centrifuged to give Pcs **7a** and **8a** as a blue/green solid. If desired, the Pcs can further be purified by running thorugh LH-20 column (see Supporting Information for ZnPc **7a**).

ZnPc 8a. Obtained as a green solid (1.9 g, 20% yield). (Yields of the asymmetrically substituted Pcs were calculated on the basis of the phthalonitrile loading, 0.1 mmol/g, of the resin **3**.) Condition A was employed to perform HPLC. t_R = 37.90 min. ESI calcd $C_{88}H_{128}N_8O_{24}S_8Zn^+$ 2003.93; found 2003.59. λ_{max}(abs) (DMSO): 709 nm (log ε = 5.5). λ_{max} (em) (DMSO): 716 nm. ϕ_f: 0.13

Selective Cleavage of Mtt Protecting Group (11). The Pc-bound polymer support was suspended in a solution of TFA/CH_2Cl_2/TIPS (2:96:2; 10 mL) and agitated for 2 min. The yellow-colored solution was filtered off. This step was repeated for 7 times using 10 mL of cleavage cocktail at a time. The resin **10** was washed with CH_2Cl_2 (10 × 10 mL) and submitted to the next step.

Bromoacetylation of Polymer-Bound ZnPc 11 (12). Polymer-bound ZnPc **11** (0.5 g) with 0.1 mmol/g Pc loading, was swelled in DMF for 30 min prior the synthesis. Following the filtration, the resin was suspended in 1 mL of DMF. Bromoacetic acid (1.1 mmol, 153 mg) and *N*,*N*′-diisopropylcarbodiimide (DIC) (1.32 mmol, 126.2 mg) were dissolved in 2 mL of CH_2Cl_2/DMF (1:1) mixture, and the solution was added to resin. The reaction was agitated for 45 min. The reaction was repeated twice. The resin was washed with DMF (6 × 15 mL), CH_2Cl_2 (3 × 15 mL), and DMF (3 × 15 mL) and used in the next reaction. Approximately 50 mg of the resin was suspended into a solution of TFA/CH_2Cl_2/TIPS (20:78:2; 1 mL) for 3 h at room temperature. The collected filtrate was evaporated to dryness, and the crude mixture was purified by filtration through a LH-20 column using CH_3OH as eluent. MALDI-MS: calcd $C_{68}H_{76}N_{11}O_{16}BrZn^+H$ 1449.72; found 1449.80.

Polymer-Bound Azide Pc 13. Polymer-bound bromoacetylated ZnPc **12** (0.5 g) was suspended in 4 mL of DMF/H_2O (5:1 v/v) solution. To the suspension was added NaN_3 (5.5 mmol, 357 mg). The microwave vessel was placed in the microwave, and the reaction was irradiated at 400 W. The temperature was ramped to 120 °C in 3 min and was held at that temperature for 45 min. Following the reaction, the resin was washed with DMF (5 × 30 mL), MeOH/H_2O (6 × 30 mL), DMF (2 × 30 mL), and CH_2Cl_2 (4 × 30 mL). ZnPc **13** was cleaved from the polymer support and purifed by employing general methods described above.

ZnPc 13. Obtained as a blue solid (11 mg, 19% overall yield). Condition B was employed to perform HPLC. t_R = 27.48 min. MALDI-MS: calcd $C_{66}H_{76}N_{14}O_{16}Zn^+H$ 1409.50; found 1409.50. λ_{max}(abs) (DMSO): 681 ± 1 nm (log ε = 5.6). λ_{max} (em) (DMSO): 691 nm. ϕ_f: 0.09

Preparation of Oligonucleotide-Pc Conjugate 9. The sequence of the 17mer oligonucleotide is 5′(aldehyde C6)-GTA AAA CGA CGG CCA GT 3′. To a 4.3 mM oligonucleotide solution in water (4.4 μL) in an Eppendorf tube were added NaOAc buffer (pH = 5.5; 50 μL), 10 mM ZnPc **7b** solution in CH_3OH (51.6 μL), and 10 mM $NaCNBH_3$ solution in CH_3OH (10 μL) in the given order. The reaction was incubated either at room temperature for 16 h or in the microwave for 30 min. The Eppendorf tube was placed in a screw-capped microwave vessel containing enough water to surround the Eppendorf tube (∼1.5 mL), and the vessel was tightened. The microwave vessel was equipped with temperature and pressure probes, and the reaction was irradiated at 400 W. The temperature was ramped to 70 °C in 1 min and held at the set temperature for 30 min. Following the completion of the reactions, cold ethanol (300 μL) and 3 M NaCl solution in water (10 μL) were added to reaction mixtures, and the suspensions were placed in −20 °C and kept for 1 h. The suspensions were centrifuged at 4 °C at 12,000 rpm for 30 min. The precipitates were reconstituted in 0.05 M triethylammonium acetate (TEAA) solution in water and isolated by HPLC in 79% labeling efficiency (76% labeling efficiency was obtained when the reaction was incubated at room temperature). Condition B was employed to perform HPLC. t_R = 18.95 min. ESI-TOF calcd (M − 3) 2255.14; found 2255.14. λ_{max}(abs) (H_2O): 640, 690 nm. λ_{max} (em) (pH 8.5 buffer; 4 mM $MgCl_2$, 15 mM KCl 10 mM TRIS) 699 nm. Unreacted oligonucleotide t_R = 1.38 min.

Preparation of Oligonucleotide-Pc Conjugate 10. The sequence of the 17mer oligonucleotide is 5′(carboxylic acid C10)-GTA AAA CGA CGG CCA GT 3′. To a 3.6 mM oligonucleotide solution in water (4.4 μL) in an Eppendorf tube were added $NaHCO_3$ buffer (pH = 7.6; 60 μL), 10 mM ZnPc **7b** solution in CH_3OH (30 μL), 150 mM 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) solution in DMSO (5 μL), and 350 mM *N*-hydroxysuccinimide (NHS) solution in DMSO (5 μL) in the given order. The Eppendorf tube was placed in a screw-capped microwave vessel containing enough water to surround the Eppendorf tube (∼1.5 mL), and the vessel was tightened. The microwave vessel was equipped with temperature and pressure probes, and the reaction was irradiated at 400 W. The temperature was ramped to 75 °C in 1 min and held at the set temperature for 30 min. Following the completion of the reaction, cold ethanol (300 μL) and 3 M NaCl solution in water (10 μL) were added to reaction mixture, and the suspension kept at −20 °C for 1 h. The suspension was centrifuged at 4 °C at 12,000 rpm for 30 min. The precipitate was reconstituted in 0.05 M TEAA solution in water and isolated by HPLC in 35% labeling efficiency. Condition B was employed to perform HPLC. t_R = 19.17 min. Unreacted oligonucleotide t_R = 1.43 min.

Preparation of Oligonucleotide-Pc Conjugate 14. The sequence of the 18mer oligonucleotide is 5′(Hexynyl C6)-GTA-AAA-CGA-CGG-CCA-AGT. To a 5 mM oligonucleotide solution in water (8 μL) in an Eppendorf tube were added 10 mM ZnPc **7b** solution in CH_3OH (120 μL), 20 mM solution of $CuSO_4 \cdot 5H_2O$ in water (24 μL), *freshly* prepared 100 mM solution of Na ascorbate in water (24 μL) and water (4 μL) in the given order (water/CH_3OH, 1:2). The Eppendorf tube was placed in a screw-capped microwave vessel containing enough water to surround the Eppendorf tube (∼1.5 mL), and the vessel was tightened. The microwave vessel was equipped with temperature and pressure probes, and the reaction was irradiated at 400 W. The temperature was ramped to 60 °C in 1 min and held at the set temperature for 60 min. Following the completion of the reaction, cold ethanol (300 μL) and 3 M NaCl solution in water (10 μL) were added to reaction mixture, and the suspension kept at −20 °C for 1 h. The suspension was centrifuged at 4 °C at 12,000 rpm for 30 min. The precipitate was reconstituted in 0.1 M TEAA solution in water and isolated by HPLC in 80% labeling efficiency. Condition B (using 0.1 M TEAA solution) was employed to perform HPLC. t_R = 17.04 min. Unreacted oligonucleotide t_R = 1.56 min.

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Supporting Information Available: Experimental procedures for the synthesis of phthalonitriles, spectroscopic data, and HPLC chromatograms for **7**−**10**, **13** and **14**. This material is available free of charge via the Internet at http://pubs.acs.org.