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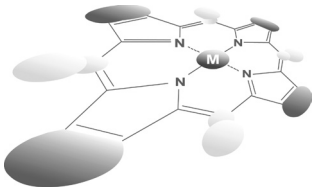

Microwave assisted solid-phase synthesis of substituted tetraazaporphyrins and a phthalocyanine-peptide conjugate

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ABSTRACT: Various asymmetrically substituted phthalocyanines (Pcs) and porphyrazines (Pzs) have been synthesized in good yields using a solid-phase synthesis method with a poly(ethylene glycol) (PEG) resin attached to an indole linker as the solid support. These compounds are formed by cross condensation of maleonitrile or phthalonitrile with another phthalonitrile covalently bonded to the solid support with an amino linking group. The polymer bound Pc or Pz is separated by filtration, and washing the symmetrical Pc or Pz by-product. The amine Pc-appended to polyethylene glycol resin is further reacted to yield azide whilst still on the solid support. Cleavage of the Pc or Pz off the solid support results in 3:1 asymmetric Pc or Pz with high degree of purity, requiring minimal further purification. The use of hydrophilic PEG-based resin allows the symmetrical compound to be removed completely by washing whereas the acid labile indole linker makes it easier to cleave the product under mild conditions. The conjugation abilities of these compounds have been demonstrated by the successful conjugation of one of the azide Pcs with a peptide elaborated with an alkyne function. Use of microwave for the synthesis of these compounds results in shorter reaction times, higher yields and higher degree of purity.

KEYWORDS: solid-phase synthesis, microwave assisted, tetraazaporphyrins, phthalocyanine-peptide conjugate, macrocyclization.

INTRODUCTION

Phthalocyanines (Pcs) and porphyrazines (Pzs) and their congeners are members of the tetraazaporphyrin (Tazp) family of aromatic macrocycles that have uniquely tunable physical, photophysical, and chemical properties, which make them suitable for a wide range of applications that range from their original use as dyes and pigments [1], to cancer therapy [2], to designer supramolecular assemblies [3] and nanotechnology [3, 4]. The synthesis and applications of Tazps have been summarized in a recent monograph [4] as well as in a number of review articles [1, 4–13]. The continued growth of interest in Tazp derivatives for so many different applications derives from the increased ability to tune the exact structure and physical properties of the porphyrinoids to suit the application. The ability to specifically design properties of Tazps is due in large part to advances in the synthetic approaches that allow control of structural elements such as the type and position of peripheral functionalization [14] variation of the central metal core [5] and symmetry/asymmetry of the porphyrinoid.

Unsymmetrically substituted Tazps (A_3B, A_2B_2, $(AB)_2$, *etc*.) are particularly useful in biological and materials applications as they allow orientation of functionality around the Tazp in a defined manner [10, 12]. A number a synthetic approaches to unsymmetrical Tazps have been developed including statistical tetramerization of two or more monomer species (*e.g.* malonitrile, phthalocyanine) [11], ring expansion of subphthalocyanines (and related structures) [13] and polymer-supported approaches in solution [7] or in the solid-phase [9]. There are a number of recent examples of the use of improved synthetic

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approaches to unsymmetrical Pcs, Pzs and related derivatives to provide compounds with improved biological or materials properties. For example, Barrett, Hoffman and coworkers reported a multigram synthesis of an A_3B porphyrazine derivative using a statistical condensation approach [15]. The same groups have made extensive use of polymerization-based capture-release synthesis of porphyrinoids [6, 7, 16] by statistical condensation of two different monomers, one containing a norbornene group that is polymerizable by ROM, which allows selective capture and subsequent release of the A_3B compounds. Torres and collaborators have utilized a statistical condensation approach to prepare an asymmetrically-substituted, monoazide-functionalized phthalocyanine that is utilized to decorate single-wall carbon nanotubes *via* Click chemistry [17]. The resulting Pc-nanotube conjugates function as a highly efficient light capturing photovoltaic cell. A number of groups are developing near-IR absorbing asymmetrically substituted Tazps as photodynamic therapy agents including recent work by Zimick and coworkers on aza-substituted porphyrazines [18], as well as developments by the groups of Barrett and Hoffman on napthyl-substitued [19] and chiral porphyrazines [20].

Our laboratory has been developing porphyrinoids as fluorescent tags for biological molecules and bioanalytical applications [21–26]. This has required addition of water-solubilizing groups as well as a site for covalent attachment of the porphyrinoids. For example, we have utilized carboxylated or pegylated Pcs for labeling of DNA oligonucleotides for both PCR-based assays and in mutation detection schemes as molecular beacons [21, 25]. Building on the precedent of Leznoff and coworkers [27, 28], we have developed a generalized solid-phase method for asymmetrically substituted Pcs, using modified Linstead cyclotetramerization approach [22, 23]. In this method one phthalonitrile group is attached to a hydrophilic, crosslinked polyethylene glycol (PEG) support and reacted with an excess of a different phthalonitrile in solution providing a 3+1 condensation product attached to the resin. This approach allows the incorporation of functional group handles into the solid-supported monomer and solubilizing groups into the three phthalonitrile subunits that add from the solution phase. The use of the hydrophilic PEG-based resin is critical to the reproducibility and high yield of the approach. Also, the utilization of microwave heating greatly reduces the time required to prepare the resin-bound tetraazaporphyrins and provides higher yields [22]. The current paper presents an expansion of this method wherein alterative linker chemistries are applied and solid-supported synthesis is applied to the synthesis of tetrazaporphyrinoids from mixtures of maleonitrile and phthalonitrile monomers. Additionally, one of the resulting asymmetrically-substituted tetraazaporphyrin containing an azide functional group is utilized in a solid-phase "Click" conjugation protocol to provide a peptide-Tazp conjugate.

RESULTS AND DISCUSSION

The retrosynthetic scheme for the tetraazaporphyrins is as shown in Fig. 1. In this case the amine phthalonitrile group is attached to a hydrophilic, crosslinked polyethylene glycol (PEG) support and reacted with an excess of a different phthalonitrile or maleonitrile in solution providing a 3+1 condensation product attached to the resin which upon cleavage provides the 3+1 asymmetric Tazp product. Conjugation can be carried out whilst the product is still on the solid support. Aminomethyl-ChemMatrix PEG resin was chosen because of the hydrophilic nature of the polymer core, which allows for complete removal of the symmetrical Tazp by washing. We also chose an aldehyde-containing linker, based on formyl indole that allows the attachment of the phthalonitrile through an amine function by reductive amination. This amine serves as diversity point for attachment of a wide range of functionalities, making the synthesis of asymmetric Pcs or Pzs with different functional groups possible. Using the amine whilst still attached to the resin, we were able to link the Pcs to the azide functionality and not much purification was required on cleavage of the Pc

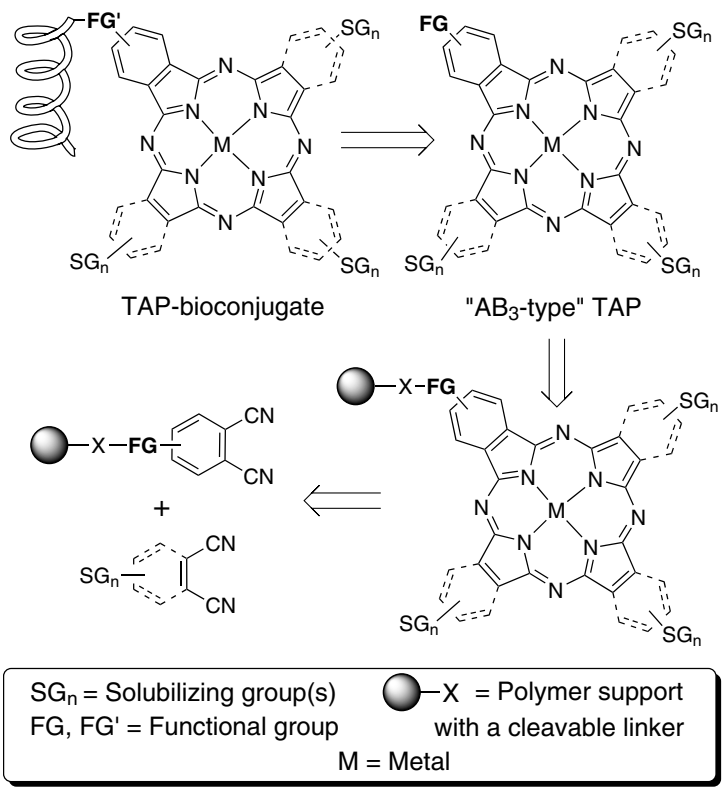


Fig. 1. Retrosynthetic scheme of the tetraazaporphyrins

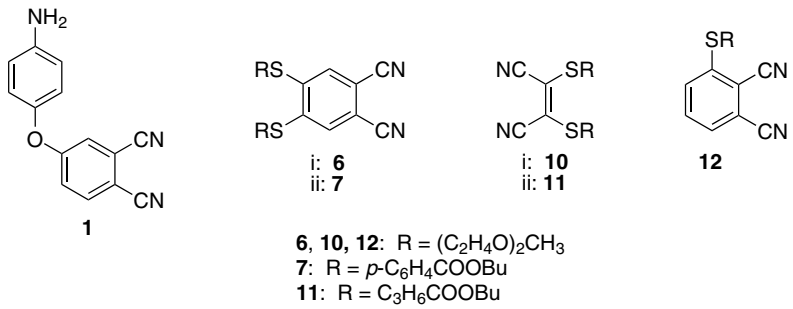


Fig. 2. Phthalonitriles and maleonitriles used in the Pcs and Pzs synthesis

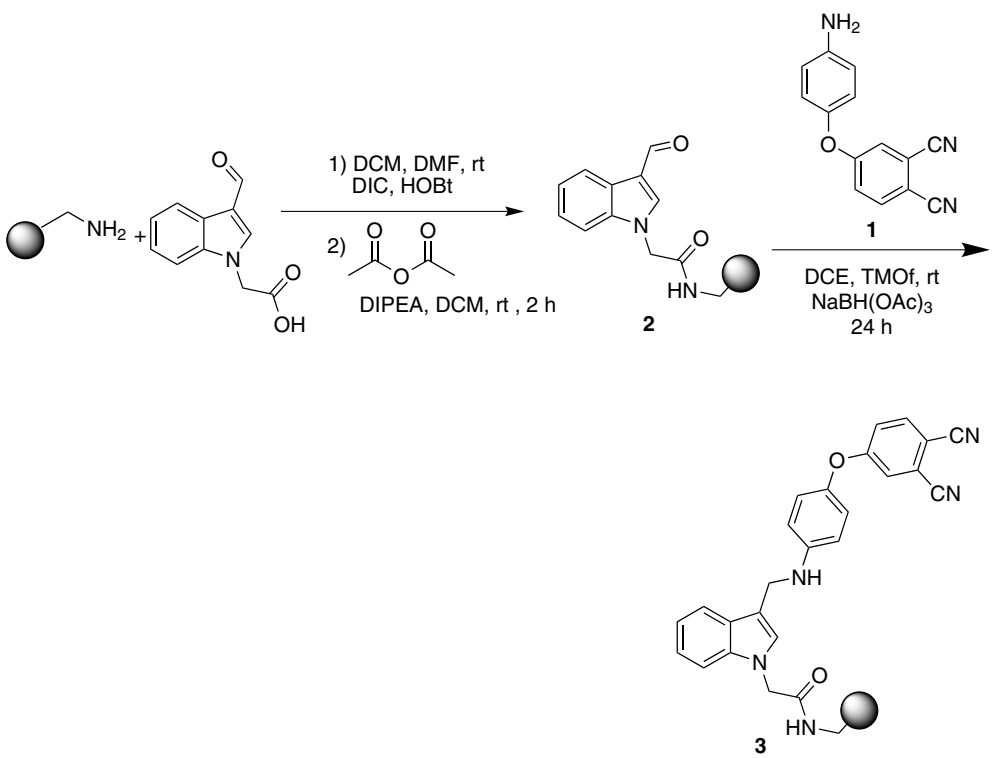
off the resin. The azide Pc on solid support was also successfully conjugated to a peptide, also making purification of the product on cleavage off the resin much easier.

The phthalonitriles and maleonitriles used for the synthesis of the Pcs and Pzs in this study are shown in Fig. 2. These were prepared using base-catalyzed nucleophilic displacement of nitro, chloride or bromide on commercially available phthalonitriles and disodium maleonitrile (Na_2MNT). See the Supporting information section for the details of their synthesis.

As depicted in Scheme 1, the polymer was attached to the indole linker, after which the amine-phthalonitrile **1**, prepared using commercially available 4-nitrophthalonitrile and *p*-hydroxyaniline in 72% yield, was covalently attached by the reductive amination method to the so-formed indole PEG resin **2** to give polymer bound phthalonitrile **3**.

The synthesis of phthalonitriles **6** was accomplished by base-catalyzed nucleophilic aromatic chloride displacement of the commercially available 4,5-dichlorophthalonitrile with monomethyl diethylene glycol thiol **4** [29–31]. Condensation of resin-bound phthalonitrile **3** with the phthalonitrile **6** in the presence of zinc acetate dihydrate, catalytic amount of DBU and 1-BuOH was achieved by microwave heating. The temperature was ramped to 180 °C in 15 min and maintained at that temperature for 1 h to give compound **13a** in solution and **13b** on solid support. The symmetrical Pc **13a** was drained, and the solid supported compound **13b** was further washed with 1-BuOH and CH_2Cl_2 until the washings were clear.

Amines bound on indole resin can easily be cleaved by 5–10% TFA/CH_2Cl_2 (4 h) with no need for a cation scavenger [32, 33]. However, we were able to increase the acid concentration in the cocktail mixture to 20–25% in the presence of a scavenger such as, triisopropyl silane, and reduced the Pc or Pz exposure to acid by 1–2 h. The crude products showed a high degree of purity with no evidence of the symmetrical compound but some of the



Scheme 1. Attachment of phthalonitrile **1** to PEG-resin bound indole linker

2:2 compound was also present as determined by MALDI MS characterization. This problem was attributed to the reaction between the polymer-bound phthalonitriles if the active sites were too close to each other as a result of low concentration of the phthalonitrile in solution or high concentration of the active sites on the starting polymer. This problem was solved by either significantly increasing the concentration of the starting phthalonitrile (30 times) or reducing the available active sites. Although both methods worked well, we settled for the reduction of the loading capacity and capping the remaining amine sites because the other method consumed a lot of the starting phthalonitrile, making it synthetically non-viable. The starting resin loading capacity of 0.6 $mmol.g^{-1}$ was reduced to 0.19 $mmol.g^{-1}$ and the remaining free amines capped with acetic anhydride in the presence of diisopropyl amine to give resin-bound phthalonitrile **3** with a reduced loading capacity. Condensation of **3** with phthalonitrile **6** in the presence of zinc(II) acetate dihydrate and 1-BuOH by microwave irradiation gave Pc **13a** in solution and the solid supported, compound **13b**. The asymmetric Pc **13c** was freed from the polymer support after washing off **13a** from the solid supported **13b** and passed through a plug of Sephadex LH-20 to remove low molecular weight impurities. The yield of this compound was 10% based on the loading of the phthalonitrile **1** on the resin. The symmetrical Pc **13a** was purified by silica column, eluting with 10% MeOH in CH_2Cl_2 to give **13a** in 17% yield. Compounds **13a** and **13c** show Q-bands above 700 nm, which is typical of 4,5-dithio-substituted compounds [34]. The fluorescent quantum yield of **13a** was found to be 0.15 and is within the expected range of similar phthalocyanines with zinc as the metal at the center [35]. The normalized absorbance and emission spectra of **13a** are shown in Fig. 3. Compound **13a** exhibits highly pronounced Q-band at 710 nm and emission band centered at 719 nm. The Q-band of **13c** is slightly blue shifted to 705 nm and has a maximum emission band at 714 nm.

Azide Pc **13d** was synthesized as depicted in Scheme 2. Compound **13b** was dried under vacuum for 24 h. Reaction of the swollen **13b** in anhydrous CH_2Cl_2 with chloroacetyl chloride in the presence of *N*,*N*-diisopropylethylamine (DIPEA) for 16 h resulted in chloro-acetamide Pc bound to the resin which was dried *in vacuo* before the nucleophilic displacement of the chloride by NaN_3 in DMSO. The azide-functionalized resin bound Pc was then subjected to the 20% TFA in CH_2Cl_2 cocktail to free the asymmetrical azide Pc **13d**. Following purification of the crude azide Pc **13d** by passing through a plug of Sephadex LH-20 to remove low molecular weight impurities, the asymmetric azide Pc **13d** was obtained in 14% yield based on the loading of the starting phthalonitrile **1** on the resin. The azide Pc **13d** has maximum absorption centered at 700 nm and maximum emission band at 712 nm.

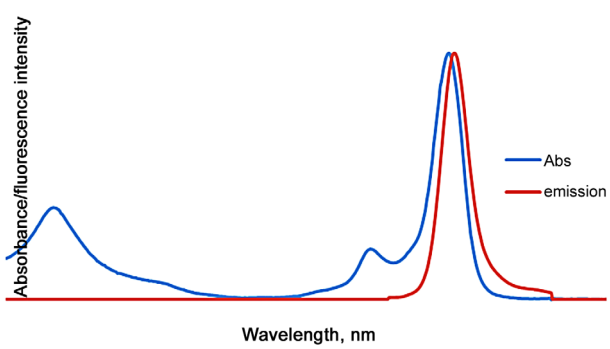
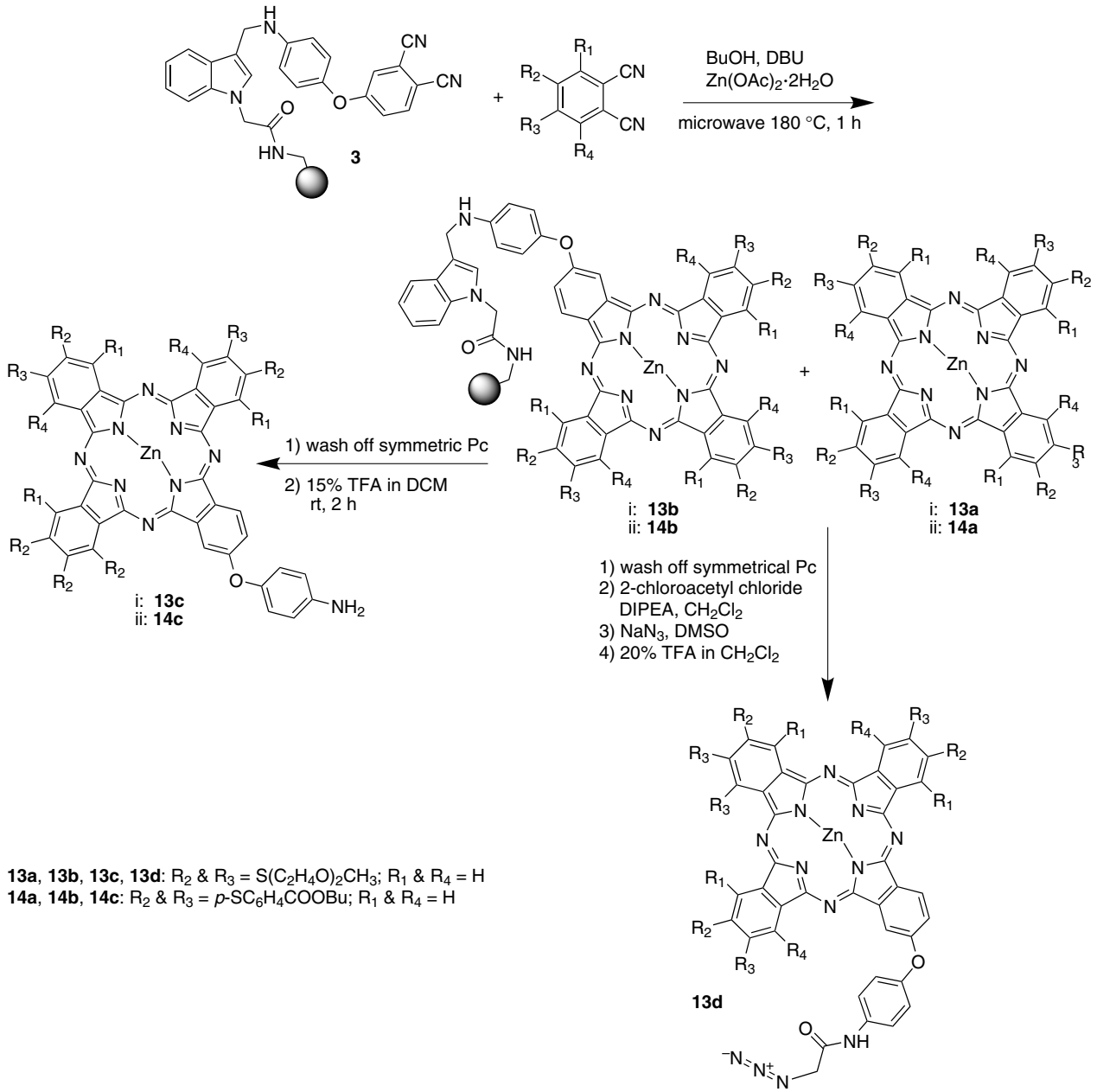


Fig. 3. Normalized absorbance and emission spectra of **13a** in DMSO

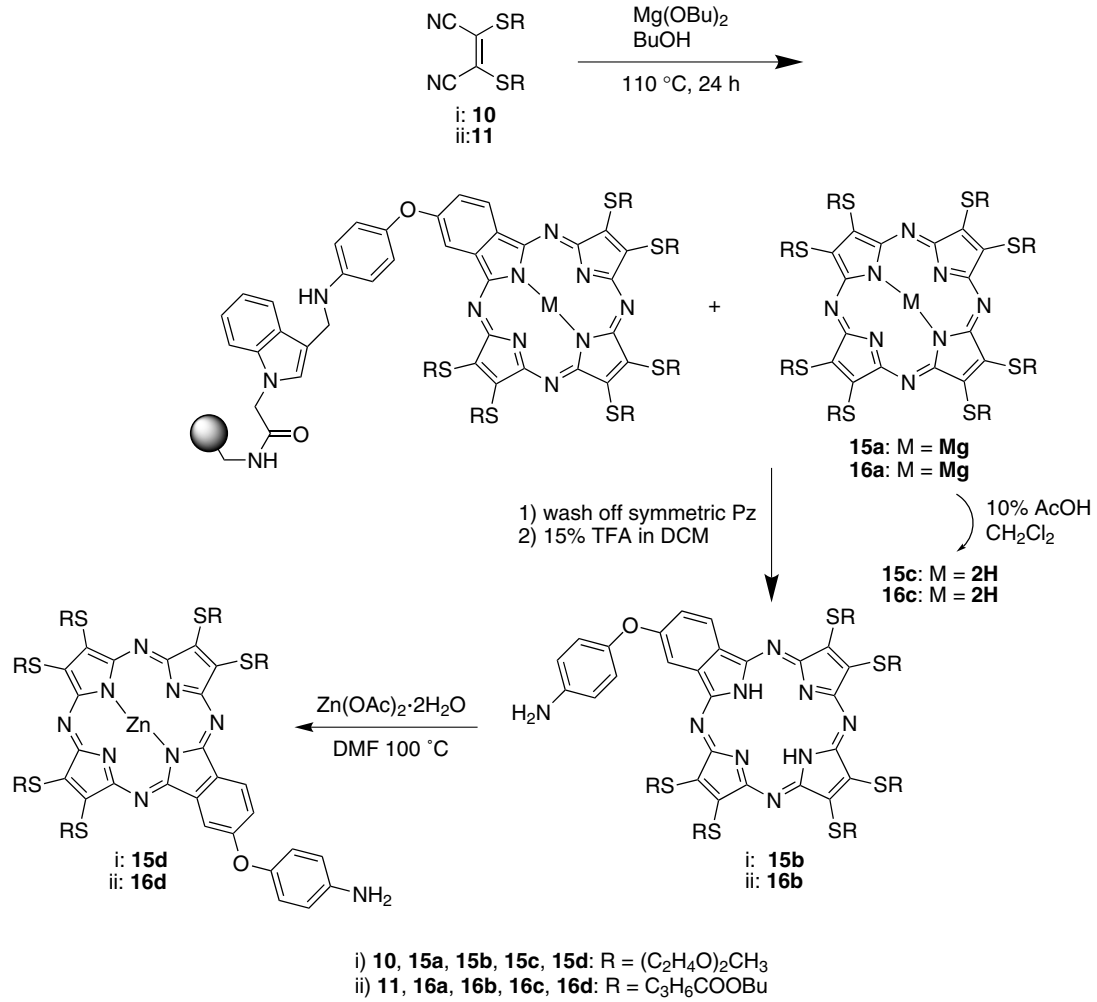
The synthesis of compounds **14a** and **14c** started by Fischer esterification of *p*-benzoic thiol with 1-butanol and H_2SO_4 as catalyst to give butyl 4-mercaptobenzoate **5**, which was used to synthesize the ester phthalonitrile **7** by base-catalyzed nucleophilic aromatic displacement of 4,5-dichlorophthalonitrile in 81% yield. As depicted in Scheme 2, macrocyclization of phthalonitrile **7** with the resin-bound phthalonitrile **3** using zinc acetate dihydrate, catalytic amount of DBU and 1-BuOH heating at 180 °C by microwave irradiation gave Pc **14a** in solution and the solid supported, compound **14b**. Following washing of **14a** and cleavage off the solid support of Pc **14c**, the compounds were further purified on a silica column in the case of **14a** and on a Sephadex LH20 column for asymmetric Pc **14c**. Pc **14a**, obtained in 19% yield, has its Q-band centered at 706 nm whereas that of **14c** with a yield of 11% is blue shifted to 697 nm.

Asymmetric Pz were also obtained by the solid support method. Disodium maleonitrile (Na_2MNT) [36] was used to synthesize maleonitrile **10** by the reaction similar to literature procedures in which 2-(2-methoxyethoxy)ethyl bromide, catalytic amount of NaI and Na_2MNT were reacted by refluxing in dry acetone for 24 h [37, 38]. Pure maleonitrile **10** was obtained as yellow viscous oil upon chromatography of the crude dark brown oil obtained after rotary evaporation of the reaction mixture on silica, eluting with diethyl ether/CH_2Cl_2 in the ratio of 2:1. Unfortunately, attempts to directly form an asymmetric zinc inserted Pz were not successful as the cleavage resulted in mixture of the correct product together with the free-base version. This was attributed to the basic nature of the Pz core which made it possible for the zinc to dissociate from the core of the Pz under acid conditions. As shown in Scheme 3, cross-over macrocyclization of maleonitrile **10** with the resin bound phthalonitrile **3**, refluxing with 1-BuOH for 24 h in the presence of $Mg(OBu)_2$ resulted in Pzs **15a** and **15b** on solid support. Following washing, cleavage, and purification, **15a** was obtained in 23% yield whereas **15b** was obtained in 11% yield. The Zn metal insertion of Pz **15b** was carried out in DMF and Zn(II) acetate at 100 °C for 24 h to obtain **15d** in yield of 9%. Treatment of **15a** with 10% acetic acid gave the free-base Pz **15c** in 17% yield.



Scheme 2. Solid-phase synthesis of symmetrical Pcs **13a** and **14a**, amine Pcs **13c** and **14c**, azide Pc **13d**

Pzs **16a** and **16b** were obtained by first converting disodium maleonitrile (Na_2MNT) [36] to maleonitrile **11**. The coupling reaction of 1-butanol and 4-bromo-butyric acid using diisopropylcarbodiimide (DIC) and diisopropylcarbodiimide (DMAP) in solvents, dichloromethane and dimethyl formamide, gave an oily suspension containing butyl 4-bromobutanoate **9** [39]. The oily suspension was filtered to remove the precipitate before further purification on a silica column, eluting with dichloromethane to give the desired product, butyl 4-bromobutanoate **9**, as yellow oil in 91% yield. Maleonitrile **11** was then obtained by the reaction of 4-bromo-butyl butanoate **9** in a reaction similar to literature procedures whereby the two reactants Na_2MNT and 4-bromo-butyl butanoate **9** were heated to reflux in dry acetone in the presence of catalytic amount of NaI for 24 h [38]. Pure maleonitrile **11** was obtained in 72% yield following flash chromatography, eluting with dichloromethane. The Pzs **16a** and **16b** were obtained by macrocyclization of resin bound phthalonitrile **3** with maleonitrile **11** in the presence of $Mg(OBu)_2$, and 1-butanol at 110 °C for 18 h to give a deep blue color of **16a** in solution and **16b** attached to the solid support. After washing of **16a**, **16b** was cleaved from the solid support using 15% TFA in CH_2Cl_2. The compounds were purified by passing each through a column of silica, eluting with 10% MeOH in ethyl acetate in both cases to give **16a** in 22% yield and **16b** in 9% yield. The Zn metal insertion of Pz **16b** was carried out using Zn(II) acetate dihydrate in the DMF at 100 °C for 24 h to obtain **16d** in yield of 8%. Treatment



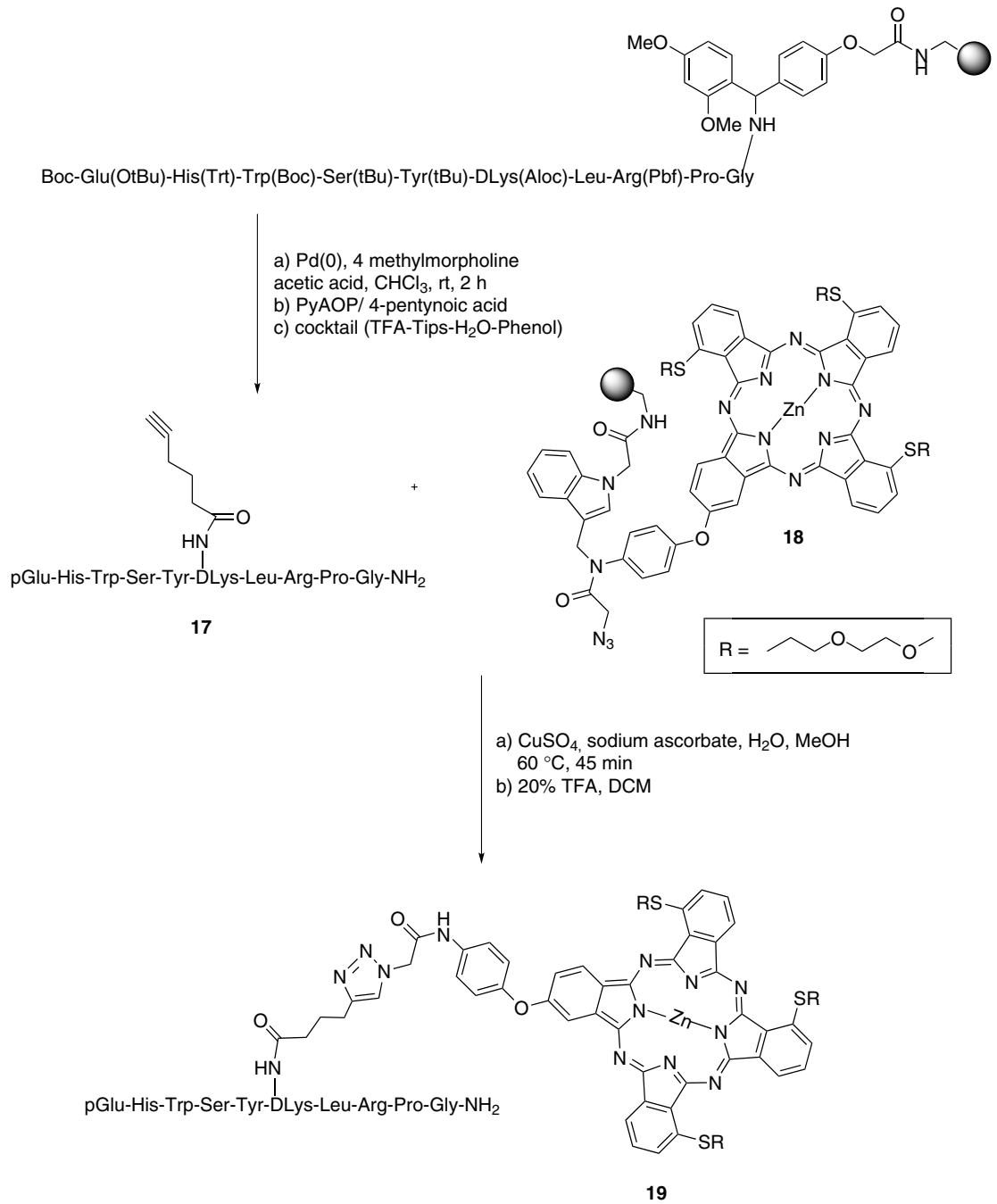
Scheme 3. Solid-phase synthesis of symmetrical Pzs **15a**, **15c**, **16a** and **16c** and amine Pzs **15b**, **15d**, **16b** and **16d**

of **16a** with 10% acetic acid gave the free-base Pz **16c** in 18% yield. The free-base compound **16c** showed a split Q-band with one centered at 670 nm and the other one at 710 nm. The compounds with the metal at the core have their Q-bands, slightly broadened around 675 nm.

The water soluble peptide **17** used for conjugation was synthesized by standard solid phase chemistry using an Fmoc-PAL-PEG-PS support on a Pioneer Peptide synthesizer (see the Supporting information section for details of synthesis).

The solid-supported azide Pc **18** was synthesized in a similar manner to the one depicted in Scheme 2 except that phthalonitrile **12**, synthesized by base-catalyzed nucleophilic aromatic nitro displacement of the commercially available 3-nitrophthalonitrile with monomethyl diethylene glycol thiol **4** [29–31] was used in place of phthalonitrile **6.** The conjugation of peptide **17** to solid supported azide Pc **18** was then carried out using 'Click chemistry' whereby the solid supported azide functionalized Pc **18** was reacted with the water soluble alkyne-functionalized peptide using a method similar to the one described by François Morvan by use of microwave irradiation [40]. The alkyne functionalized water soluble peptide **17**, the solid-supported azide Pc **18**, copper(II) sulfate, sodium ascorbate, methanol and water were all transferred into a microwave vial. The vial was sealed and irradiated with microwave at 60 °C for 45 min. Treatment of the resin beads with a cocktail of TFA/CH_2Cl_2 (1:4) for 2 h at room temperature afforded the triazole conjugate in 31% yield. The conjugate was characterized by ESI mass spectrometry and purity verified by HPLC, carried out using the gradient elution with TEAA, MeOH and THF. Compound **19** is readily soluble in MeOH and is partially soluble in a MeOH/H_2O solution, an indication of great improvement on the solubility properties of the Pc.

All Pcs and Pzs were characterized by HPLC, MALDI, and absorbance and fluorescence spectroscopy. The yields of all asymmetrical compounds were between 8–14% based on the loading of the phthalonitrile bound to the resin, whilst those of the symmetrical compounds were between 17–23% as shown in Table 1. Except for compounds **15b**, **15d**, **16b** and **16d**, which were purified on a silica column, all asymmetric compounds were purified on Sephadex LH20 column, whereas all symmetric ones were purified on a silica column. These compounds



Scheme 4. Conjugation of PEG resin-bound azide Pc **18** to alkyne peptide **17**

are highly soluble in most common organic solvents such as EtOAc, CH_2Cl_2, $CHCl_3$, DMSO, DMF, THF, and MeOH. The increased solubility is due to the periphery substitution of the macrocycles with solubilizing groups that is ethylene glycol, ester, amine and azide groups. Except for the octa-thio butyl ester substituted compound **14a**, which was taken in THF; all electronic spectra were taken in DMSO. The spectra for Pcs are typical of metallated Pcs, characterized by narrow Q-bands as an indication of monomeric behavior in DMSO [41]. The HOMO and LUMO energy gap is reduced by introducing sulfur as well as free nitrogen at the periphery of macrocycles resulting in compounds absorbing well in the red (703–710 nm). Compound **13a** shows a Q-band above 700 nm, which is typical of 4,5-dithio-substituted compounds [34]. Porphyrazines exhibit pronounced B-band between 370 and 400 nm and a split Q-band at 643 and 710 nm for free-base ester Pz **16c**. The splitting is caused by loss of degeneracy of the lowest unoccupied molecular orbitals. The band between 400–550 nm can be ascribed to S(n) to π^* transitions [37]. Free-base as well as metallated asymmetrical porphyrazines showed broadened Q-bands typical of Pzs of reduced symmetry [42, 43]. Fluorescence quantum yields were determined by a comparative

Table 1. Compound properties

Compound	Yield, %	Solvent	Q-band λ_{max}, nm	Log ε, $cm^{-1}{\cdot}M^{-1}$	Emission λ_{max}, nm	Stokes shift, nm	Φ_F
13a	17	DMSO	710	5.2	719	9	0.15
13c	10	DMSO	705	5.1	714	9	0.051
13d	14	DMSO	700	5.5	712	12	0.12
14a	19	THF	705	5.5	716	11	0.15
14c	11	DMSO	703	5.1	713	10	0.049
15a	23	DMSO	675	5.1	*n.d.*	—	*n.d.*
15b	11	DMSO	675	4.6	*n.d.*	—	*n.d.*
15c	17	DMSO	674	4.8	*n.d.*	—	*n.d.*
15d	9	DMSO	675	4.7	*n.d.*	—	*n.d.*
16a	22	DMSO	675	4.9	*n.d.*	—	*n.d.*
16b	9	DMSO	674	4.7	*n.d.*	—	*n.d.*
16c	18	DMSO	670	4.7	*n.d.*	—	*n.d.*
16d	8	DMSO	675	4.7	*n.d.*	—	*n.d.*

method [44] using methylene blue (Φ_F = 0.03) [45] in methanol as the standard. All fluorescence measurements were performed in solutions with absorbances of 0.047–0.054 for both Pc and standard with excitation ranging from 630 nm to 665 nm depending on the absorbances of the Pcs. Emission recorded in all cases refers to the Q-band emission. Fluorescence quantum yields of the compounds are within the normal range for those compounds [46]. The Pzs did not show any fluorescence when excited near the Q-band region. Whilst the sulfur and nitrogen's n to π* transitions contribute to the absorbance in the red, the atoms appear to cause rapid decay of the excited states through radiationless conversion to sulfur and nitrogen (n, π*) states as well as the ground states [37], hence reducing the quantum yields. This is particularly pronounced in asymmetrical molecules bearing the sulfur as well as the free amine group at the periphery. The properties of the compounds are summarized in Table 1.

EXPERIMENTAL

See the Supporting information section for the general experimental procedures and the synthesis of phthalonitriles and maleonitriles.

Polymer-bound N-ethyl-2-(3-formyl-indol-1-yl)acetamide (2). Aminomethyl PEG resin (3.0 g, 0.66 $mmol.g^{-1}$) was swelled in dry CH_2Cl_2 (36 mL) for 30 min in a 100 mL two neck round bottom flask, with one neck sintered. To the swollen resin was added dry DMF (4 mL), 4-formyl indoyl acetic acid (0.1341 g, 60 mmol), HOBt (0.26 g, 1.98 mmol) and DIC (0.2329 g, 1.85 mmol). The mixture was shaken under argon at room temperature for 24 h. The resin was drained, washed with DMF (2 × 30 mL), MeOH (2 × 30 mL), and CH_2Cl_2 (2 × 30 mL) respectively. The resin was then dried under vacuum after which the remaining amine functions were capped by first swelling the resin in CH_2Cl_2 (20 mL) for 15 min and then adding dry diisopropyl ethyl amine DIPEA (12 mL) and acetic anhydride (8 mL) to the swollen resin and shaking the mixture at room temperature for 2 h. The solvents were then drained and the resin further washed with DMF (2 × 30 mL), MeOH (2 × 30 mL) and CH_2Cl_2 (2 × 30 mL), respectively. The resin was then dried under vacuum overnight to give the indole functionalized PEG resin **2**.

Reductive amination of polymer-bound N-ethyl-2-(3-formyl-indol-1-yl)acetamide (3). Polymer-bound *N*-ethyl-2-(3-formyl-indol-1-yl)acetamide (4.027 g, 0.886 mmol) was swelled in dry DCE (40 mL) for 15 min in a 100 mL two neck round bottom flask, with one neck sintered. Trimethylorthoformate (20 mL), the amine phthalonitrile **1** (2.08 g, 8.84 mmol) and $NaBH(OAc)_3$ (1.88 g, 8.87 mmol) were added to the swollen resin. The mixture was shaken at room temperature for 16 h, after which the solvents were drained, and resin was washed with DMF (2 × 20 mL), MeOH (2 × 20 mL) and CH_2Cl_2 (2 × 20 mL) respectively. The resin was then dried under vacuum for 24 h.

Determination of loading capacity of resin 3. Polymer bound *N*-ethyl-2-(3-formyl-indol-1-yl)acetamide **2** (0.2 g) was suspended in dry dichloroethane (DCE) (4 mL) for 15 min in a 50 mL two neck round bottom flask, with one neck sintered. Trimethyl orthoformate (2.0 mL), mono-Fmoc 1,3-diaminopropane hydrochloride (0.2 g, 0.60 mmol) and $NaBH(OAc)_3$ (0.127 g, 0.60 mmol) were added to the swollen resin. The mixture was shaken at room temperature for 16 h, after which the solvents were drained, and resin was washed with DMF (2 × 10 mL), MeOH (2 × 10 mL) and CH_2Cl_2 (2 × 10 mL) respectively to give the Fmoc-amino acid resin. The resin was then dried under vacuum and then 5.1 mg of the dried resin was shaken in piperidine:DMF (3:7) (0.5 mL) for 30 min, following which MeOH (6.5 mL) was added and the resin filtered off. For reference, piperidine:DMF:MeOH (0.3:0.7:39) solution was prepared. Spectrophotometric

analysis was carried out at 301 nm, with comparison to a free Fmoc Ala of known concentration treated under similar conditions. The loading capacity of the resin **3** was found to be 0.19 mmol.g^{-1}.

Octa- and hexa-substituted-[2-(2-methoxy-ethoxy) ethylsulfanyl]Pcs (13a and 13c). The dried resin bound amine phthalonitrile **3** (0.5622 g, 0.123 mmol), 4,5-[2-(2-methoxy-ethoxy)ethyl sulfanyl]-phthalonitrile **6** (0.8 g, 3.053 mmol) and zinc acetate dihydrate (0.2 g, 0.91 mmol) were added into a dry microwave reaction vessel purged with argon. 1-butanol (15 mL) was added to the mix. The mixture was heated in a water bath to swell the resin and dissolve the phthalonitrile as well as the zinc acetate. To this reaction mixture was added catalytic amount of DBU (0.1 g) before the reaction was purged again with argon and microwave vessel sealed. The microwave temperature was ramped to 180 °C in 15 min. The reaction temperature was held at 180 °C for 1 h to complete the reaction. After cooling to 50 °C the contents in the microwave were poured into a three neck round bottom flask, with one neck sintered and the symmetrical Pc **13a** drained leaving the resin bound, compound **13b** in the flask. Compound **13b** was then washed, first with hot 1-butanol, a solution of 1-butanol/CH_2Cl_2, and CH_2Cl_2 until washings were clear and the symmetrical Pc **13a** completely removed. The washings containing the symmetrical Pc **13a** were combined, rotary evaporated at room temperature to give a green solid which was further purified by column chromatography on a silica column, eluting with 10% MeOH in CH_2Cl_2. The symmetrical Pc **13a** was obtained in (0.19 g, 17%) yield. The resin supported, compound **13b** was then treated with a cocktail of CH_2Cl_2 (12 mL, TFA (3.5 mL) and triisopropylsilane (0.3 mL). This mixture was shaken at room temperature for 2 h to cleave the polymer bound Pc **13c**. The resin was then drained and washed 3 more times with CH_2Cl_2 (5 mL) to completely remove the cleaved Pc **13c**. The filtrate was evaporated using rotary evaporator at room temperature to give a green solid. Chromatography of the crude Pc **13c** on a Sephadex LH 20 column, eluting with 10% MeOH in CH_2Cl_2 gave the desired product Pc **13c**. **Pc 13a.** Yield 0.19 g (17%). UV-vis-NIR (DMSO): λ_{max}, nm 710. MS (MALDI-TOF): *m/z* 1648.43 (calcd. for $[M + H]^+$ $C_{72}H_{96}N_8O_{16}S_8Zn$ 1648.4). HPLC retention time (t_R = 43.19 min). Φ_F: 0.15. **Pc 13c.** Yield 18 mg (10%). UV-vis-NIR (DMSO): λ_{max}, nm 705. MS (MALDI-TOF): *m/z* 1487.38 (calcd. for $[M + H]^+$ $C_{68}H_{81}N_9O_{13}S_6Zn$ 1487.36). HPLC retention time (t_R = 42.93 min). Φ_F: 0.051.

Asymmetric azide Pc (13d). Compound **13b** (0.8 g, 0.18 mmol) was swelled in dry CH_2Cl_2 (15 mL) for 15 min. To this swelled resin was added dry DIPEA (0.37 mL, 2.12 mmol) followed by dropwise addition of chloroacetyl chloride (0.123 mL, 1.54 mmol). The mixture was shaken under argon for 14 h after which the resin was drained, washed with dry DMF (2 × 20 mL), MeOH (2 × 30 mL) and CH_2Cl_2 (2 × 30 mL) respectively. The resin was then dried under vacuum to give acetyl chloride bound resin. The acetyl chloride bound resin (0.8 g, 0.18 mmol) was swelled in DMSO for 30 min. To this swelled resin was added sodium azide (1.5 g, 2.31 mmol). The mixture was stirred on a shaker at room temperature for 48 h after which it was washed with distilled water (2 × 50 mL), DMF (2 × 20 mL), and CH_2Cl_2 (2 × 30 mL) respectively. The resin was dried *in vacuo*. The dried azide bound resin (0.8 g, 0.18 mmol) was swelled in CH_2Cl_2 (5 mL). To the swelled resin was added a cocktail of TFA/CH_2Cl_2 (1 mL:4 mL). The mixture was shaken for 2 h. The resin was drained and washed with CH_2Cl_2 (5 × 10 mL). The washings were combined and evaporated at room temperature to give a dark green solid. The solid was purified by chromatography on Sephadex LH 20 column, eluting with 10% MeOH in CH_2Cl_2 to give the azide Pc **13d** as a dark green solid. **Pc 13d.** Yield 40 mg (14%). UV-vis-NIR (DMSO): λ_{max}, nm 700. MS (MALDI-TOF): *m/z* 1570.41 (calcd. for $[M + H]^+$ $C_{70}H_{82}N_{12}O_{14}S_6Zn$ 1570.37). Φ_F: 0.12. HPLC retention time (t_R = 43.43 min).

Octa- and hexa-substituted thio-butyl benzoate Pcs (14a and 14c). A mixture of 4,5-(4-mercaptobenzoic butyl ester)phthalonitrile **7** (1.25 g, 2.31 mmol), dried resin bound amine phthalonitrile **3** (0.7 g, 0.154 mmol), zinc acetate dihydrate (0.32 g, 1.36 mmol) in dry 1-butanol (15 mL) was heated in a two neck round bottom flask connected to a condenser under argon to 90 °C before catalytic amount of DBU (0.1 g) was added to the mixture. Heating continued for 24 h, maintaining the temperature at 110 °C. During this period the mixture changed from pale yellow to green and finally dark green. The solution containing **14a** was drained, leaving the resin bound, compound **14b** in the flask. Compound **14b** was then washed to completely remove the symmetrical, first with hot 1-1-BuOH, mixture of 1-BuOH/CH_2Cl_2, and CH_2Cl_2 until the washings were clear. The washings were combined, rotary evaporated, and further purified on silica column eluting with 10% MeOH in CH_2Cl_2 to give the symmetrical Pc **14a** in (0.197 g, 19%) yield. Compound **14b** was treated with a cocktail of 5 mL TFA, 0.2 mL triisopropylsilane and 19.8 mL CH_2Cl_2 for 2 h to cleave the Pc **14c**. The solution containing the cleaved Pc **14c** was drained; the resin washed and drained three more times to remove all the cleaved Pc, **14c** from the resin. The combined washings were rotary evaporated at room temperature to give a dark green solid which was purified by chromatography on LH 20 column, eluting with 10% MeOH in CH_2Cl_2 to give the asymmetrical Pc **14c**. **Pc 14a.** Yield 0.197 g (19%). UV-vis-NIR (THF): λ_{max}, nm 705. MS (MALDI-TOF): *m/z* 2240.3 (calcd. for $[M + H]^+$ $C_{120}H_{112}N_8O_{16}S_8Zn$ 2240.53). HPLC retention time (t_R = 47.3 min). Φ_F: 0.15. **Pc 14c.** 29.7 mg (11%). UV-vis-NIR (DMSO): λ_{max}, nm 703. MS (MALDI-TOF): *m/z* 1931.5 (calcd. for $[M + H]^+$ $C_{104}H_{93}N_9O_{13}S_6Zn$ 1931.45). HPLC retention time (t_R = 45.83 min). Φ_F: 0.049.

Octa- and hexa-substituted-[2-(2-methoxy-ethoxy) ethylsulfanyl]Pzs (15a and 15b). Magnesium turnings

(0.1, 4.1 mmol) and 0.01 g of I_2 were suspended in 1-butanol (20 mL) in microwave vessel. The microwave temperature was ramped to 180 °C in 5 min. The reaction temperature was held at 180 °C for 1 h to form $Mg(OBu)_2$. After cooling to 50 °C the contents in the microwave were poured into a round bottom flask, with one of the necks sintered. The flask was connected to a condenser and purged with argon. Maleonitrile **10** (2.17 g, 6.26 mmol), resin bound amine phthalonitrile **3** (0.7 g, 0.15 mmol) were added and the suspension heated at 110 °C for 24 h. During the heating period, the reaction mixture turned yellow, and then gradually turned deep blue. After 24 h, the symmetrical Pz **15a** in solution was removed by draining. The resin remaining in the flask was washed first with hot 1-butanol (10 × 10 mL), 1-butanol/CH_2Cl_2 mix (10 × 10 mL), MeOH (5 × 10 mL) and CH_2Cl_2 (10 × 10 mL) until the washings became clear. The washings were combined, rotary evaporated to give a dark blue solid which was further purified by column chromatography on silica eluting with 10% MeOH/CH_2Cl_2 to give the symmetrical Pz **15a**. The Pz bound resin was dried *in vacuo*, swelled in CH_2Cl_2 (10 mL) for 10 min. To the swollen resin triisopropylsilane (2–3 drops) and a cocktail of TFA/CH_2Cl_2 (1 mL:4 mL) were added. The treated resin was shaken at room temperature for 30–45 min after which the free unsymmetrical Pz **15b** was drained. The resin was washed 3 more times with CH_2Cl_2 (3 × 10 mL) to completely remove the cleaved Pz. The washings were combined, rotary evaporated at room temperature to give a dark blue solid which was further purified by column chromatography on silica eluting with 10% MeOH/CH_2Cl_2 to give the unsymmetrical Pz **15b**. **Pz 15a**. Yield 0.47 g (23%). UV-vis (DMSO): λ_{max}, nm 675. MS (MALDI-TOF): *m/z* 1408.29 (calcd. for $[M + H]^+$ $C_{56}H_{88}MgN_8O_{16}S_8$ 1408.39). HPLC retention time (t_R = 44.35 min). **Pz 15b**. Yield 21 mg (11%). UV-vis (DMSO): λ_{max}, nm 675. MS (MALDI-TOF): *m/z* 1275.15 (calcd. for $[M + H]^+$ $C_{56}H_{77}N_9O_{13}S_6$ 1275.4). HPLC retention time (t_R = 42.62 min).

Octa-substituted-[2-(2-methoxy-ethoxy)ethylsulfanyl]Pz (15c). A mixture of Mg Pz **15a** (1.2 g, 0.85 mmol), and 10% AcOH (6 mL) in dry CH_2Cl_2 (5 mL) was stirred under argon at room temperature for 2 h. To this mixture was added ammonium hydroxide dropwise until the solution was basic. The mixture was poured into a separating funnel and washed with water. The organic layer was collected, dried with $MgSO_4$ after which it was rotary evaporated to give crude **15c** which was further purified on silica column to give Pz **15c**. **Pz 15c**. Yield 0.82 g (17%). UV-vis (DMSO): λ_{max}, nm 674. MS (MALDI-TOF): *m/z* 1386.4 (calcd. for $[M + H]^+$ $C_{56}H_{90}N_8O_{16}S_8$ 1386.42). HPLC retention time (t_R = 42.48 min).

Hexa-substituted-[2-(2-methoxy-ethoxy)ethylsulfanyl]Pz (15d). A mixture of asymmetric Pz **15b** (1.2 mg, 0.000093 mmol) and Zn(II) acetate dihydrate (0.013 g, 0.00093 mmol) in dry DMF (5 mL) was heated in a 25 mL round bottom flask at 90 °C for 24 h under argon. The blue solution was then rotary evaporated to give crude Pz **15d**. The crude **15d** was purified on silica column using 10% MeOH in CH_2Cl_2 to give **15d** in (0.99 mg, 9%) yield based on the starting phthalonitrile on the resin. **Pz 15d**. Yield 0.99 mg (9%). UV-vis (DMSO): λ_{max}, nm 675. MS (MALDI-TOF): *m/z* 1337.28 (calcd. for $[M + H]^+$ $C_{56}H_{75}N_9O_{13}S_6Zn$ 1337.31). HPLC retention time (t_R = 42.54 min).

Octa- and hexa-substituted thio-butyl butanoate Pzs (16a and 16b). Magnesium turnings (0.10 g, 4.1 mmol) and 0.01 g of I_2 were suspended in 1-butanol (20 mL) in microwave vessel. The microwave temperature was ramped to 180 °C in 15 min. The reaction temperature was maintained at 180 °C for 1 h to form $Mg(OBu)_2$. After cooling to 50 °C the contents in the microwave were poured into a round bottom flask with one neck sintered. The flask was connected to a condenser and purged with argon. Maleonitrile **11** (2.82 g, 6.26 mmol), resin bound amine phthalonitrile **3** (0.7 g, 0.15 mmol) were added into the flask and the suspension heated at 110 °C for 24 h. During the heating period, the reaction mixture turned yellow, and then gradually turned deep blue. After 24 h, the symmetrical Pz **16a** in solution was removed by draining. The resin attached to the asymmetric Pz **16b** was washed first with hot 1-butanol, 1-butanol/CH_2Cl_2, MeOH and CH_2Cl_2 until the washings became clear. The washings were combined, rotary evaporated to give a dark blue solid which was further purified by column chromatography on silica, eluting with 5% MeOH in CH_2Cl_2 to give the symmetrical Pz **16a**. The Pz bound resin was dried *in vacuo*, swelled in CH_2Cl_2 (10 mL) for 10 min. To the swollen resin triisopropyl silane (2–3 drops) and a cocktail of TFA:DCM (1 mL:4 mL) were added. The treated resin was shaken at room temperature for 30–45 min after which the free unsymmetrical Pz **16b** was drained. The resin was washed 3 more times with (3 × 10 mL) CH_2Cl_2 to completely remove the cleaved Pz. The washings were combined, rotary evaporated at room temperature to give a dark blue solid which was further purified by column chromatography on silica eluting with 5% MeOH in CH_2Cl_2 to give the unsymmetrical Pz **16b**. **Pz 16a**. Yield 0.55 g (22%). UV-vis (DMSO): λ_{max}, nm 675. MS (MALDI-TOF): *m/z* 1728.56 (calcd. for $[M + H]^+$ $C_{80}H_{120}MgN_8O_{16}S_8$: 1728.64). HPLC retention time (t_R = 45.37 min). **Pz 16b**. Yield 20 mg (9%). UV-vis (DMSO): λ_{max}, nm 674. MS (MALDI-TOF): *m/z* 1515.7 (calcd. for $[M + H]^+$ $C_{74}H_{101}N_9O_{13}S_6$: 1515.58). HPLC retention time (t_R = 42.36 min).

Octa-substituted thio-butyl butanoate Pzs (16c). A mixture of Mg Pz **16a** (1.5 g, 0.87 mmol), and 10% AcOH in dry CH_2Cl_2 (12 mL) was stirred under argon at room temperature for 2 h. To this mixture ammonium hydroxide was added dropwise until the solution was basic. The mixture was poured into a separating funnel and washed with water. The organic layer was collected, dried with $MgSO_4$ after which it was rotary evaporated to give crude Pz **16c** which was further purified on silica

column, eluting with 10% MeOH in CH_2Cl_2 to give the title compound. **Pz 16c**. Yield 1.21 g (18%). UV-vis (DMSO): λ_{max}, nm 670. MS (MALDI-TOF): *m/z* 1706.68 (calcd. for $[M + H]^+$ $C_{80}H_{122}N_8O_{16}S_8$: 1706.67). HPLC retention time (t_R = 42.9 min).

Hexa-substituted thio-butyl butanoate Pz (16d). A mixture of asymmetric Pz **16b** (1.1 mg, 0.000073 mmol) and $Zn(OAc)_2{\cdot}2H_2O$ (0.013 g, 0.00093 mmol) in dry DMF (5 mL) was heated in a 25 mL round bottom flask at 90 °C for 24 h under argon. The blue solution was then rotary evaporated to give crude Pz **16d**. The crude **16d** was purified on silica column using 5% MeOH in EtOAc to give **16d** in (0.95 mg, 8%) yield based on the starting phthalonitrile on the resin. **Pz 16d**. Yield 0.95 mg (8%). UV-vis (DMSO): λ_{max}, nm 675. MS (MALDI-TOF): *m/z* 1577.47 (calcd. for $[M + H]^+$ $C_{74}H_{99}N_9O_{13}S_6Zn$: 1577.5). HPLC retention time (t_R = 44.3 min).

Polymer-bound asymmetric azide Pc (18). The dried resin bound amine phthalonitrile **3** (0.6 g, 0.123 mmol), phthalonitrile **12** (1.5 mmol, 0.4 g), 1-butanol and zinc(II) acetate dihydrate (0.3 g, 1.37 mmol) were added to the microwave reaction vessel purged with argon. The mixture was heated in a water bath to swell the resin and dissolve the phthalonitrile as well as the zinc acetate dihydrate. The reaction mixture was purged again with argon and the reaction vessel was closed. The microwave temperature was ramped to 180 °C in 15 min. The reaction temperature was held at 180 °C for 1 h to complete the reaction. After cooling to 50 °C the contents in the microwave were poured into a round bottom flask, with one neck sintered and the symmetrical Pc drained leaving the resin bound Pc in the flask. The resin bound Pc was washed first with hot 1-BuOH, mixture of CH_2Cl_2/1-BuOH and CH_2Cl_2 until the washings were clear. The resin was then dried under vacuum for 24 h. The resin bound to the amine Pc was swelled in dry CH_2Cl_2 (15 mL) for 15 min. To this swelled resin dry DIPEA (0.37 mL, 2.12 mmol) was added, followed by dropwise addition of chloroacetyl chloride (0.123 mL, 1.54 mmol). The mixture was shaken under argon for 14 h after which the resin was drained, washed with dry DMF (2 × 20 mL), MeOH (2 × 30 mL) and CH_2Cl_2 (2 × 30 mL) respectively. The resin was then dried under vacuum to give acetyl chloride bound resin. The acetyl chloride bound resin was swollen in DMSO before adding sodium azide (1.5 g, 2.31 mmol). The mixture was stirred on a shaker at room temperature for 48 h after which it was washed with distilled water (2 × 50 mL), DMF (2 × 20 mL), and CH_2Cl_2 (2 × 30 mL) respectively to give the resin bound to the azide Pc, compound **18**.

Azide Pc-peptide conjugate (19). Solid-supported azide Pc **18** (0.2 g, 0.05 mmol), alkyne functionalized water soluble peptide **17** (0.25 g, 0.23 mmol), copper(II) sulfate (9 mg, 0.009 mmol), sodium ascorbate (0.036 g, 0.045 mmol) methanol (6.0 mL), and water (6.0 mL) were transferred into a microwave vial. The vial was sealed and irradiated with microwave at 60 °C for 45 min. The contents of the microwave were then placed in a two neck flask, with one of the necks sintered. The solvents were drained and the remaining contents washed with MeOH (2 × 20 mL) and CH_2Cl_2 (2 × 30 mL) to remove the unreacted peptide. Treatment of the resin beads with a cocktail of TFA:CH_2Cl_2 (1:4) for 2 h at room temperature enabled the triazole conjugate which was further purified on a Sephadex LH20 column, eluting with 100% MeOH to give the azide-peptide conjugate **19** in (3 mg, 31%). The compound was characterized by ESI mass spectrometry and purity verified by HPLC. ESI-MS: *m/z* 2519.89 (calcd. for $[M + H]^+$ $C_{119}H_{143}N_{30}O_{23}S_3Zn$ 2519.94). HPLC retention time (t_R = 41.1 min).

CONCLUSION

We have successfully synthesized several asymmetrical Pcs and Pzs which contain not only solubilizing groups but also labile functional groups namely the amine and azide groups appropriate for tagging compounds with carbonyl, acid, active ester and alkyne functionalized groups, DNA or peptides for example. The conjugation abilities of these compounds have been demonstrated by the successful conjugation of one of the azide Pcs with an alkyne peptide. The conjugate is highly soluble in MeOH, an improvement of solubility properties of the Pc.

Because these compounds fluoresce in the near-IR region of the spectrum, have high molar absorption properties and high fluorescence quantum yields, their bio-conjugates can potentially be used in fluorescence detection for analysis of nucleic acids, proteins and other biological targets such as tumors [21, 25, 47–49]. These compounds could also find application as photosensitizers for the photodynamic therapy of tumors and as antiviral agents for blood sterilization. Use of microwave for the synthesis of these compounds allowed us to shorten the reaction times from 24 h to 1 h and the compounds so obtained had a high degree of purity. The use of this mild solid support synthesis method and ready-post TAP assembly functionalization will make it possible to synthesize many other important functional groups for tagging.

Supporting information

A full list of general experimental procedures, synthesis of phthalonitriles and maleonitriles, 1D proton and carbon NMR of phthalonitriles and maleonitriles and HPLC chromatograms of Pcs, Pzs, peptide and conjugate are given in the supplementary material. This material is available free of charge *via* the Internet at http://www.worldscinet.com/jpp/jpp.shtml.

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