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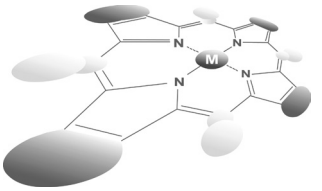

Design and synthesis of protoporphyrin IX/vitamin B_{12} molecular hybrids *via* CuAAC reaction

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ABSTRACT: The design and synthesis of new molecular hybrids composed of protoporphyrin IX (PPIX) and vitamin B_{12} via copper catalyzed alkyne azide cycloaddition reaction is described. New, clickable aminoazide and aminoalkyne linkers were prepared and subsequently attached to PPIX (*via* vinyl group) and to vitamin B_{12} giving desired building blocks. Preliminary results showed that respective water soluble hybrids were formed under CuAAC reaction. Gratifyingly, Cu incorporation into the PPIX core was avoided, which was important for further biological studies.

KEYWORDS: CuAAC, click chemistry, azides, alkynes, vitamin $B_{12,}$ protoporphyrin IX, molecular hybrids.

INTRODUCTION

For many years, nitroglycerine and other organic nitrates have been used for treatment of cardiovascular disorders, particularly coronary heart diseases, heart failure and hypertension. Such drugs release nitric oxide (NO) by spontaneous decomposition or bioconversion. This key signaling molecule is involved in the regulation of a variety of biological and physiological processes in mammals including blood pressure control and neurotransmission *via* activating soluble guanylate cyclase (sGC) [1]. The enzyme is activated by NO which binds to the heme moiety in the regulatory domain. Formation of nitrosyl heme disrupts the axial bond with histidine 105, which leads to conformational changes in the structure of the enzyme causing a dramatic increase of its activity. The NO-activated enzyme catalyses the transformation of guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP), an increased level of cGMP results in vasodilatation and inhibition of platelet aggregation [2]. However, prolonged administration of NO-releasing drugs causes many unwanted side effects, and effectiveness of the therapy decreases due to the growing tolerance [1]. Some new NO-independent activators of sGC have already been introduced and their action depends on the heme in the regulatory domain of the enzyme, similarly to NO [3].

Ignarro and coworkers showed that some corrin and tetrapyrrole compounds, in particular protoporphyrin IX (**1**, PPIX), strongly regulate sGC in *in vitro* studies, however not *in vivo* [4]. It was proposed that porphyrin **1** interacts with the regulatory domain of sGC causing its activation by replacing heme and inducing structural changes similar to those occurring after complexation to NO. It was also reported that the propionic acid groups of porphyrin **1** are crucial as they interact with tyrosine 135 and arginine 139 of sGC [5]. Dicyanocobinamide, another corrin activator discovered by Martin *et al.,* unlike other sGC regulators interacts directly with the catalytic domain of sGC by a mechanism of activation which is still unknown [6].

Thanks to the existence of highly efficient dietary uptake system in mammals [7], vitamin B_{12} has been utilized as a delivery vehicle for several bioactive and imaging molecules [8]. In particular, bioconjugates of vitamin B_{12} have been recently used in efficient oral delivery of insulin [8(h),8(j)] as well as anti-cancer drugs [8(r)] and in cancer diagnostics [8(l),8(p)]. Since the uptake system involves a series of transport proteins, conjugates of vitamin B_{12} must be recognized by them.

◊SPP full member in good standing

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It has been shown that recognition of vitamin B_{12} by the proteins, involved in its uptake (haptocorrin, intrinsic factor and transcobalamin II (TC-II)), is not impaired only when it is modified at specific sites, such as 1) 5′-hydroxy group on the ribose unit, 2) the phosphate unit, and 3) the ε peripheral propionamide unit. In the B_{12}-TC-II complex the "tail" of vitamin B_{12}, containing ribose ring and phosphate group is the only part of the B_{12} molecule accessible for the solvent [7(b)].

We envisaged that vitamin B_{12} could also facilitate uptake of PPIX and as such increase its bioavailability. Therefore, a hybrid molecule [9] composed of PPIX and vitamin B_{12} moieties was designed. Herein, we describe the synthesis of linker molecules suitable for linking vitamin B_{12} to PPIX (**1**), the synthesis of "clickable" vitamin B_{12} and PPIX derivatives, and finally our attempts to prepare their water-soluble bioconjugates.

RESULTS AND DISCUSSION

Design of the molecular hybrids

PPIX and its analogs can regulate sGC activity *via* binding to the regulatory domain. It was postulated that PPIX binds to the NO-dependent heme binding site. Moreover, the propionic acid groups at positions 2 and 18 form electrostatic bonds with amino acids' basic groups (for example arginine) and such interactions are critical for the enzyme activation [5]. Therefore, it would be optimal to incorporate PPIX into the hybrid molecule by linking it to vitamin B_{12} *via meso*-position or one of its vinyl groups. We chose the latter possibility as we thought that it would not affect binding of PPIX to the enzyme, and because methods for functionalization of PPIX at vinyl groups are known [10].

On the other hand, the role of vitamin B_{12} fragment would be to deliver the whole hybrid from the intestine to endothelium cells. Thus, it should be linked to PPIX in such a way that neither molecule obstructs each other and that does not interfere with vitamin B_{12} binding to proteins. We chose the 5′-OH group as it can be readily functionalized in a selective manner [8(b),8(p)]. The other option would involve the synthesis of ε-acid *via* mild statistical hydrolysis [7].

The final step that is connection of PPIX (**1**) to vitamin B_{12} derivative could be achieved using the copper-catalyzed azide alkyne cycloaddition reaction (CuAAC) [11], which has been already used in the preparation of various tetrapyrroles derivatives [12]. The designed approach required the synthesis of linkers with either terminal azide or alkyne moieties and on its other terminus the amino group allowing its attachment either to PPIX or vitamin B_{12} at positions specified above. The strategy is outlined in Fig. 1.

One potential difficulty with this method lay with the incorporation of copper into porphyrins which was very likely to occur under typical CuAAC conditions. Protection of the PPIX core by metalation with, for example, Zn would increase the number of synthetic steps and subsequent Zn removal, performed on the final hybrid, might be problematic. Therefore, we concentrated on finding such conditions for CuAAC under which Cu incorporation into the porphyrin core would be avoided.

Synthesis of aminoazide linkers

Various aminoazide linkers were synthesized starting either from terminal dibromides or ethylene glycols. Selective monosubstitution of terminal leaving groups in symmetrical 1,6-dibromohexane

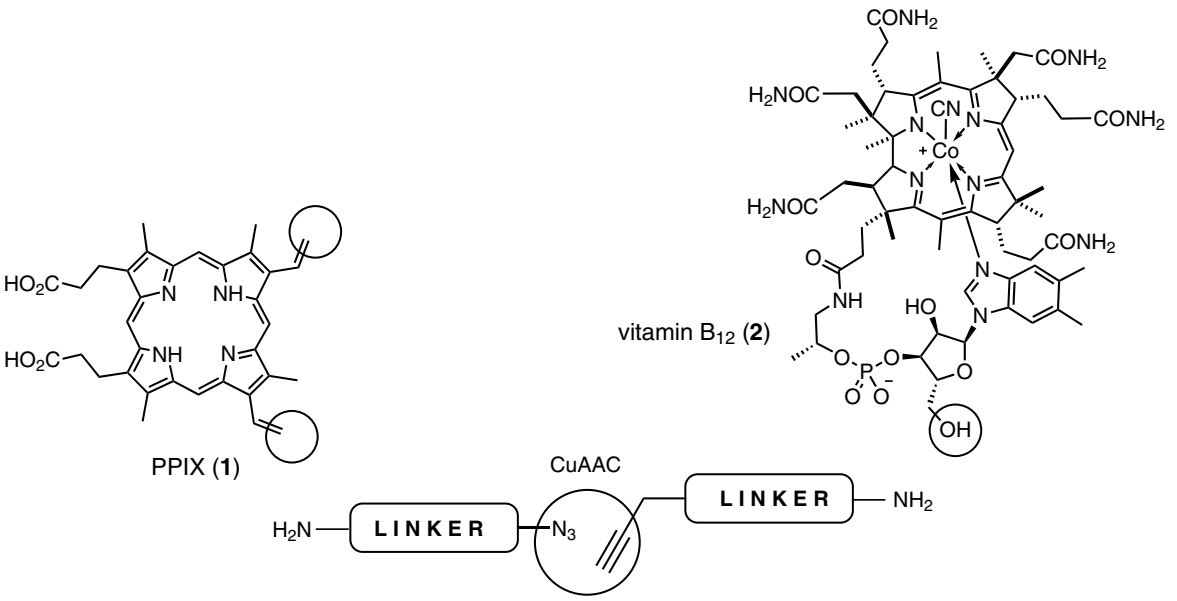
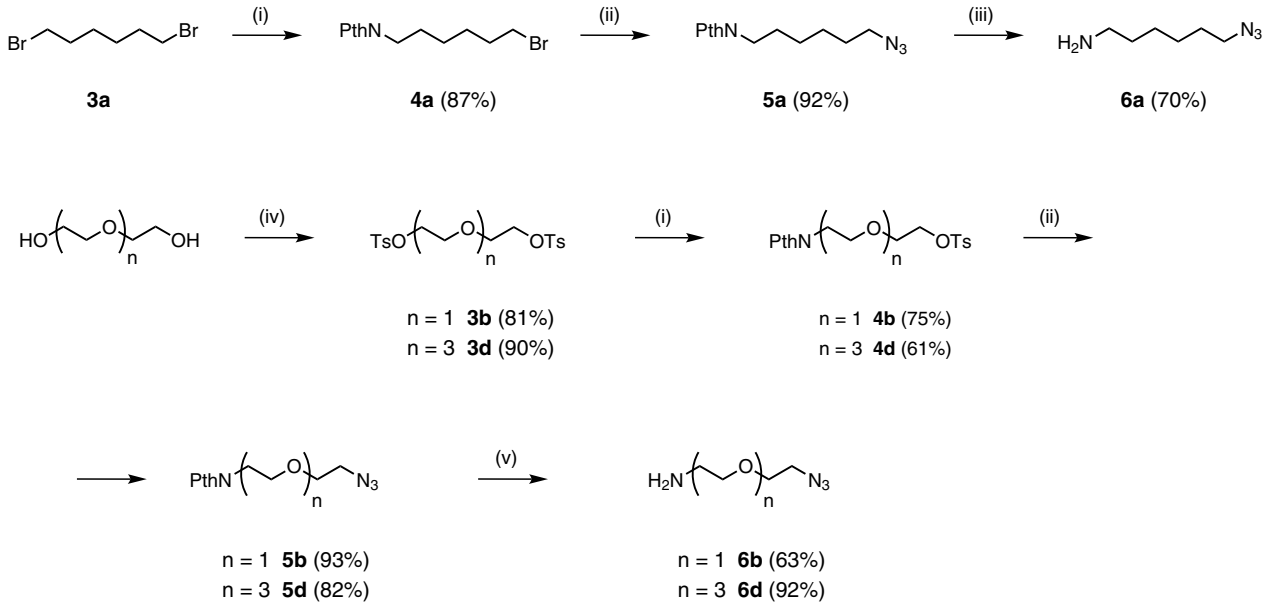
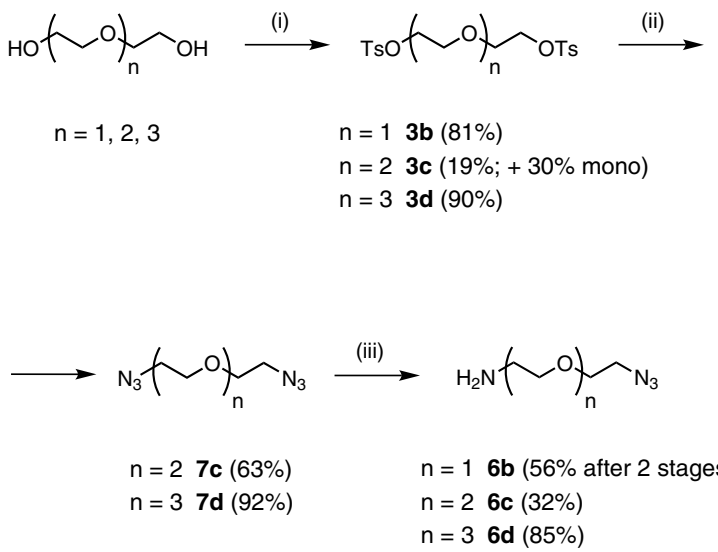


Fig. 1. The general strategy of preparation of molecular hybrids containing PPIX (**1**) and vitamin B_{12} (**2**)



Scheme 1. (i) PthNH, K_2CO_3, DMF, rt; (ii) NaN_3, DMF, 60 °C; (iii) H_2NNH_2, EtOH/H_2O, 60 °C; (iv) TsCl, NaOH, THF/H_2O, 0 °C to rt; (v) $MeNH_2$, EtOH, reflux



Scheme 2. (i) TsCl, NaOH, THF/H_2O, 0 °C to rt (for n = 1, 3) or TsCl, Py, CH_2Cl_2, 0 °C to rt (for n = 2); (ii) NaN_3, DMF, 60 °C; (iii) Ph_3P, CH_2Cl_2/THF/2M $HCl_{(aq)}$

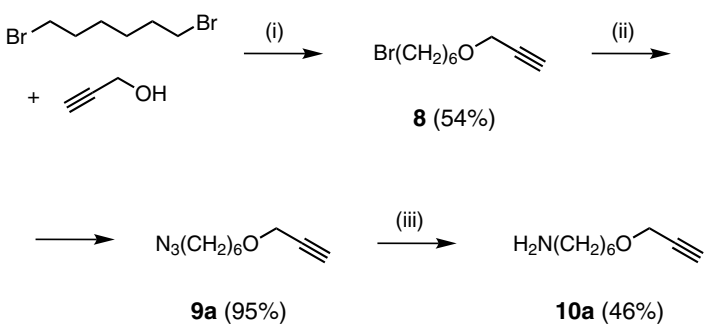
(**3a**) or glycol derivatives **3b** and **3d** with phthalimide followed by the introduction of the azide group at the other terminus gave protected amines **5** in good yield. Treatment of phtalimide derivatives **5a**, **5b**, and **5d** with methylamine afforded desired aminoazide linkers **6** (Scheme 1).

The simplified synthesis involved the Staudinger reaction as a crucial step. Bis(tosyl) derivatives **3b–3d** were reacted with sodium azide furnishing (bis)azides **7**. Subsequent, selective reduction of only one azide group led to desired amines **6** possessing a terminal azide group (Scheme 2) [13].

Synthesis of aminoalkyne linkers

The simplest alkyne linker used in this work was propargylamine. A longer chain analog could be obtained by the selective alkylation reaction of 1,6-dibromohexane (**3a**) with propargyl alcohol. Subsequent substitution of -Br with -N_3 group, using standard conditions, gave azide **9a**. The labile azidoalkyne was directly reduced with triphenylphosphine in the presence of water to afford aminoalkyne **10a** in 46% yield (Scheme 3).

A more efficient approach to aminoalkyne linkers, which avoided the low-yielding desymmetrization step and allowed to prepare the unstable alkyneazides **9** under milder conditions, involved introduction of



Scheme 3. (i) NaH, DMF, rt; (ii) NaN_3, DMF, rt; (iii) Ph_3P, THF/CH_2Cl_2/H_2O, rt

the azido group as the first step, followed by the alkylation of azidoalcohols **12** with propargyl bromide, preferably under PTC conditions with tetra-*n*-butylammonium bromide (TBAB) as a catalyst. Reduction of azide **9** to a primary amine was again accomplished under Staudinger conditions. Starting monotosylates **11** of 1,ω-diols were available through standard tosylation in NaOH/H_2O/THF system with one equivalent of TsCl (Scheme 4).

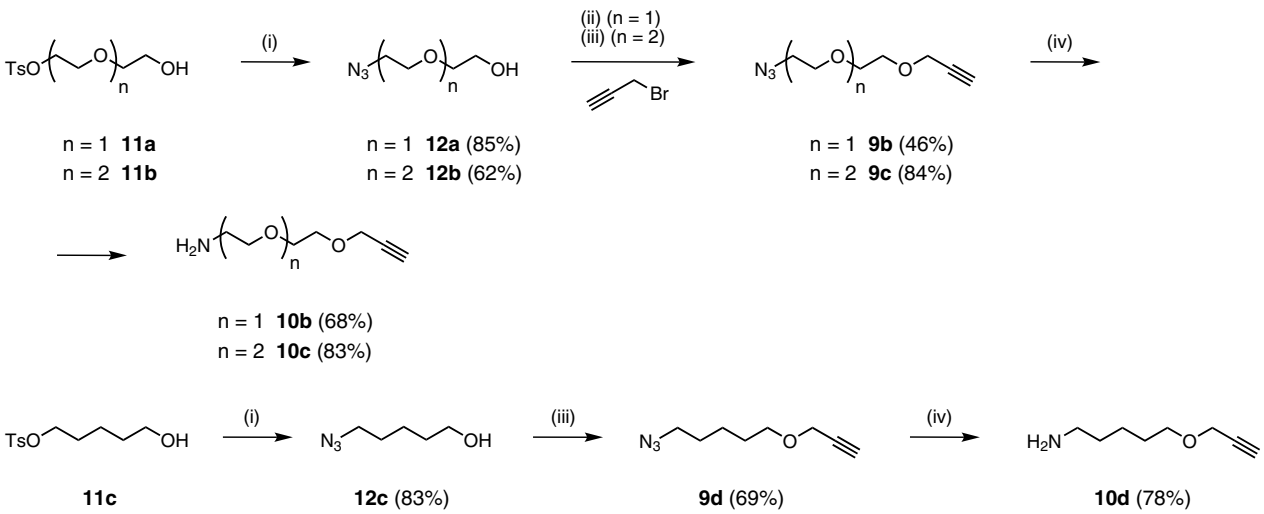
Synthesis of PPIX derivatives with terminal azide and alkyne linkers

Linkers **6** and **10** were attached to PPIX (**1**) by oxidizing one or two vinyl groups of PPIX to the aldehyde and subsequent reductive amination reaction.

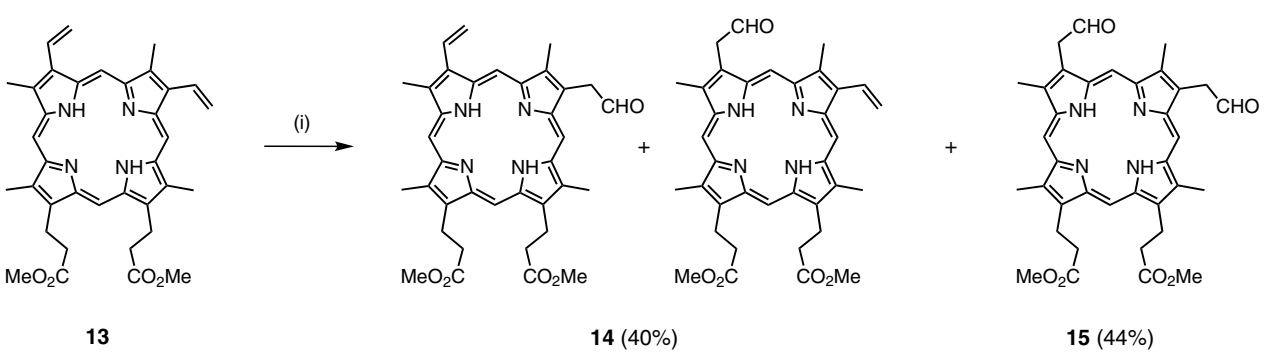
Attempts to oxidize free PPIX (**1**) resulted in its decomposition therefore dimethyl ester **13** was used as a starting material. Oxidation of protoporphyrin IX ester **13** with thallium(III) nitrate leads to PPIX bis(aldehyde). This approach is one of the most convenient methods for the synthesis of this molecule [14], but it has been also reported to provide some quantities of monoaldehydes. From our point of view such monoaldehydes would be more desirable, though bis(aldehyde) would, in theory, allow to attach two molecules of vitamin B_{12} to one porphyrin.

The reaction of protoporphyrin dimethyl ester (**13**) with $Tl(NO_3)_3{\cdot}3H_2O$ first gave dimethyl acetal derivatives which after hydrolysis with formic acid afforded a mixture of two isomeric, inseparable monoaldehydes **14** and bis(aldehyde) **15**. Under optimal conditions desired monoaldehydes **14** were obtained in 40% combined yield and bis(aldehyde) **15** in 44% yield (Scheme 5). All attempts to increase the yield of monoaldehydes **14** by decreasing the amount of $Tl(NO_3)_3{\cdot}3H_2O$ or lowering the reaction temperature led to a very low conversion of the substrate while an increase in the amount of oxidant resulted in the predominant formation of bis(aldehyde) **15**.

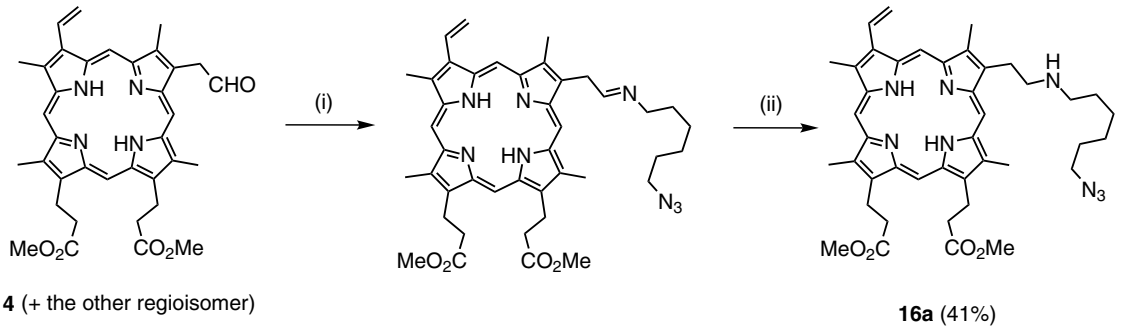
A mixture of monoaldehydes **14**, after separation from bis(aldehyde) **15**, was subjected to reductive amination with linkers **6**. First attempts were performed as follows: aldehydes **14** were stirred with an amine and a drying agent (Na_2SO_4) in CH_2Cl_2 to form two isomeric imines; after filtration and evaporation, crude imines were reduced with $NaBH_4$ in MeOH. This procedure provided porphyrin derivative **16a** in a reasonable yield (Scheme 6).



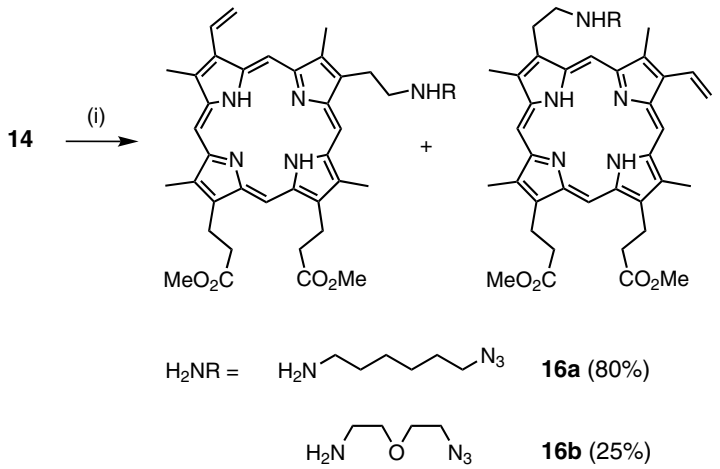
Scheme 4. (i) NaN_3, DMF, rt; (ii) NaH, DMF, rt (iii) 50% $NaOH_{(aq)}$, TBAB, toluene; (iv) Ph_3P, THF/CH_2Cl_2/H_2O, rt



Scheme 5. Oxidation of porphyrin **13**. (i) (1) 2.2 equiv. $Tl(NO_3)_3{\cdot}3H_2O$, (2) conc. $HCl_{(aq)}$, (3) HCO_2H



Scheme 6. Reductive amination of PPIX monoaldehydes **14**. (i) **6a**, Na_2SO_4, CH_2Cl_2; (ii) $NaBH_4$, CH_2Cl_2/MeOH, 0 °C to rt



Scheme 7. Reductive amination of PPIX monoaldehydes **14**. (i) RNH_2, $NaBH_3CN$, AcOH, MeCN

However, the above procedure did not work well with linkers other than **6a** and even in that case, the yields were not reproducible. A much more reliable protocol employed $NaBH_3CN$ as a reducing agent (Scheme 7) [15].

PPIX aldehydes **14** and **15** are moderately stable and their separation was troublesome. For these reasons, in terms of yield, as well as the number of laboratory operations needed and solvents and materials used, the overall efficiency of the synthesis of "clickable" porphyrin building blocks was further improved. When PPIX aldehydes **14** and **15** were not separated and purified, but instead the crude reaction mixture after oxidation and hydrolysis was used directly in the reductive amination reaction the overall yield increased. Using this protocol several PPIX derivatives of type **16** and **17** were obtained, containing both azide and acetylene linkers (Scheme 8).

Overall yields, based on ester **13**, were moderate, but they include 3 reaction steps: oxidation, cleavage of the acetal and reductive amination. Unlike aldehydes **14** and **15**, products **16** and **17** were reasonably stable and could be fairly easily separated from each other and characterized. Regioisomers of porphyrins **16** containing one linker and one vinyl group could not be separated. In some cases product **17** with two linkers was not even observed. Its yield could be increased by using 3.3 equivalents of $Tl(NO_3)_3{\cdot}3H_2O$ instead of 2.2.

Other methods of attaching linkers to PPIX (**1**) through its vinyl groups were considered as well, but they proved unsuccessful or inefficient. Alkene metathesis reaction of ester **13** or its zinc complex with Cbz-protected 8-amino-1-octene gave only traces of unidentified product while the Wittig reaction of a mixture of aldehydes **14** and **15** with an ylide stabilized with an amide group led to the decomposition of the starting material.

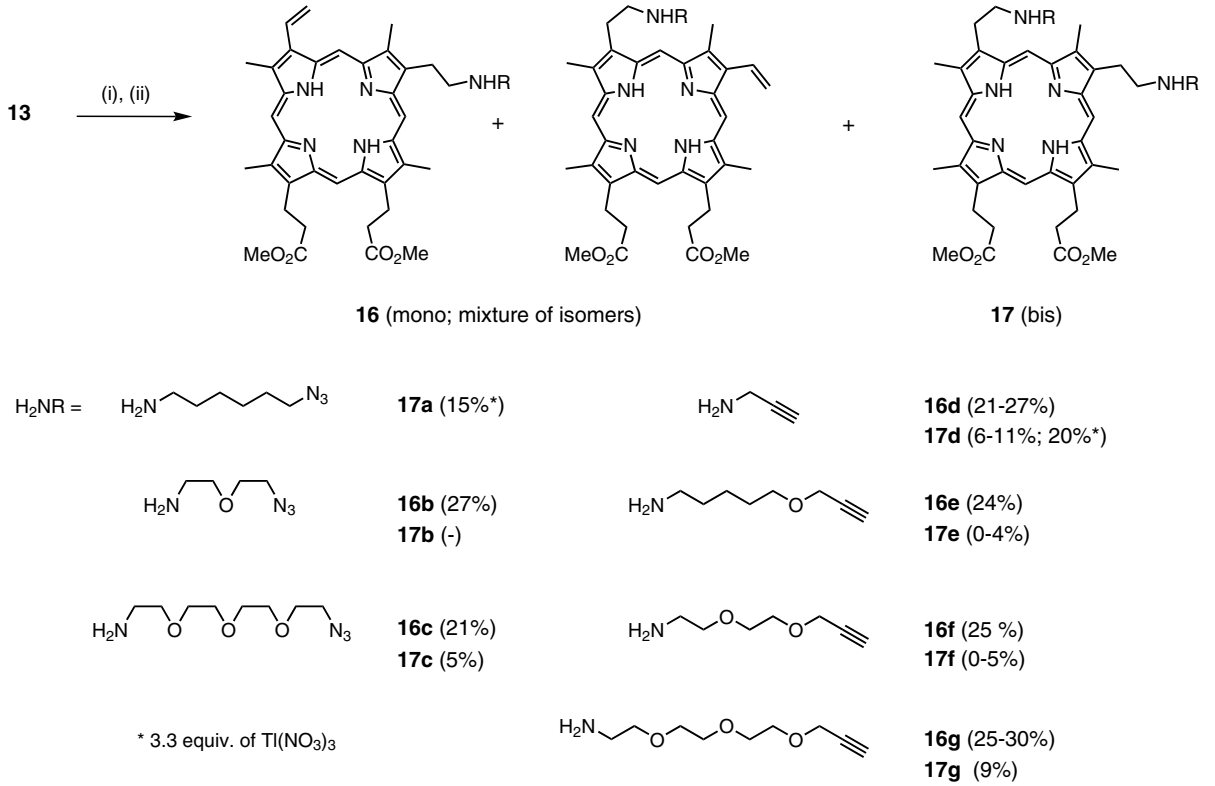
Preparation of vitamin B_{12} derivatives with azide and alkyne linkers at 5' position

Linker molecules were attached to the 5′ hydroxyl group of the ribose ring of vitamin B_{12} using the well-established methodology of carbamate formation mediated by carbonyldiimidazole (CDI) or carbonylditriazole (CDT) [8(b),(p)]. In the first reaction propargylamine and aminoalkyne **10c** gave derivatives **18a** and **b** in 35 and 38% yield respectively. A similar reaction of **10c** but using CDT instead of CDI gave 82% of **18b**. Therefore, the former coupling reagent was used in other examples of azide- and alkyne-functionalized vitamins **18** (Scheme 9).

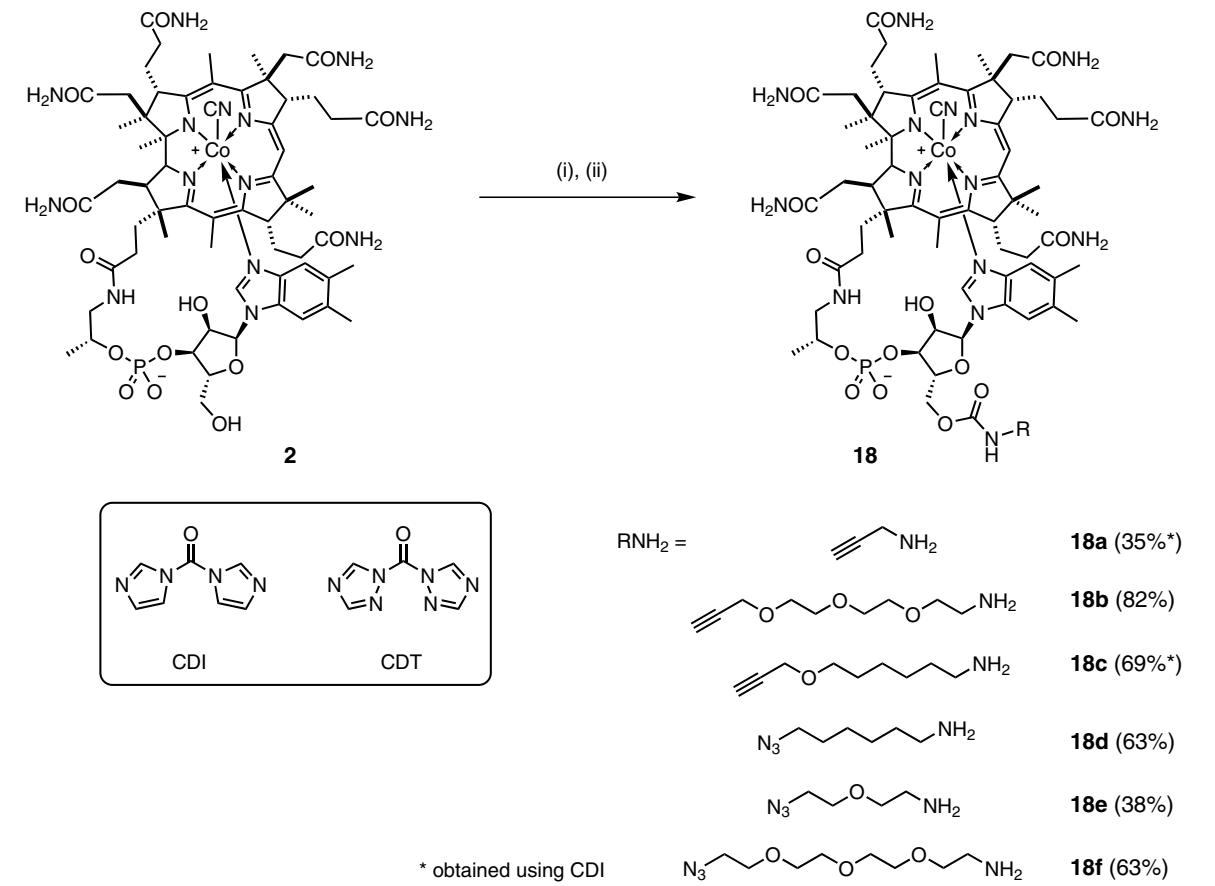
These new derivatives **18** were easily separated from small-molecule organic compounds (mainly DMSO after the reaction) by precipitation from a mixture of Et_2O with $CHCl_3$ or CH_2Cl_2 [8(p)] and washing with Et_2O. Further purification involved reverse-phase chromatography and then another precipitation and washing with Et_2O. Purity of amines **18** was confirmed using reverse-phase HPLC. ^{1}H NMR and HPLC proved that the reaction was selective at the 5' primary hydroxyl group (products of the reaction with 2′ hydroxyl were not observed).

Attempted synthesis of hybrid molecules

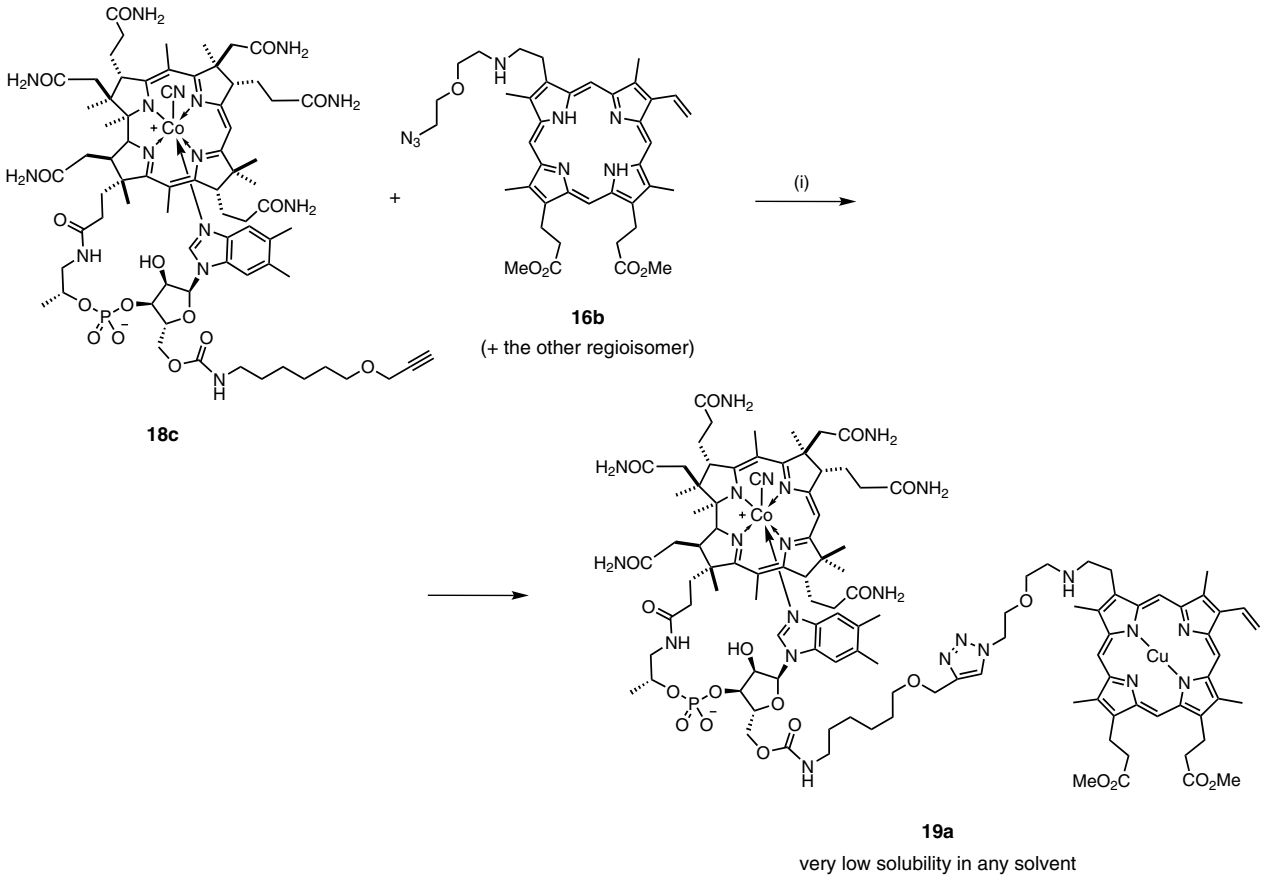
The first attempt of the CuAAC reaction of porphyrin azide **16b** with vitamin B_{12} derived alkyne **18c** (Scheme 10) was performed under standard conditions: $CuSO_4{\cdot}5H_2O$, sodium ascorbate, TBTA (tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine), t-BuOH/H_2O [11(a)]. The



Scheme 8. Preparation of PPIX derivatives with linkers (i) (1) 2.2 equiv. $Tl(NO_3)_3{\cdot}3H_2O$, (2) conc. $HCl_{(aq)}$, (3) HCO_2H; (ii) linkers **6** or **10**, $NaBH_3CN$, AcOH, MeCN



Scheme 9. Preparation of vitamin B_{12} derivatives with azide and alkyne linkers: (i) 1.5 equiv. CDT, DMSO, 40 °C, 30 min. (ii) 3.3 equiv. RNH_2, rt, 24 h

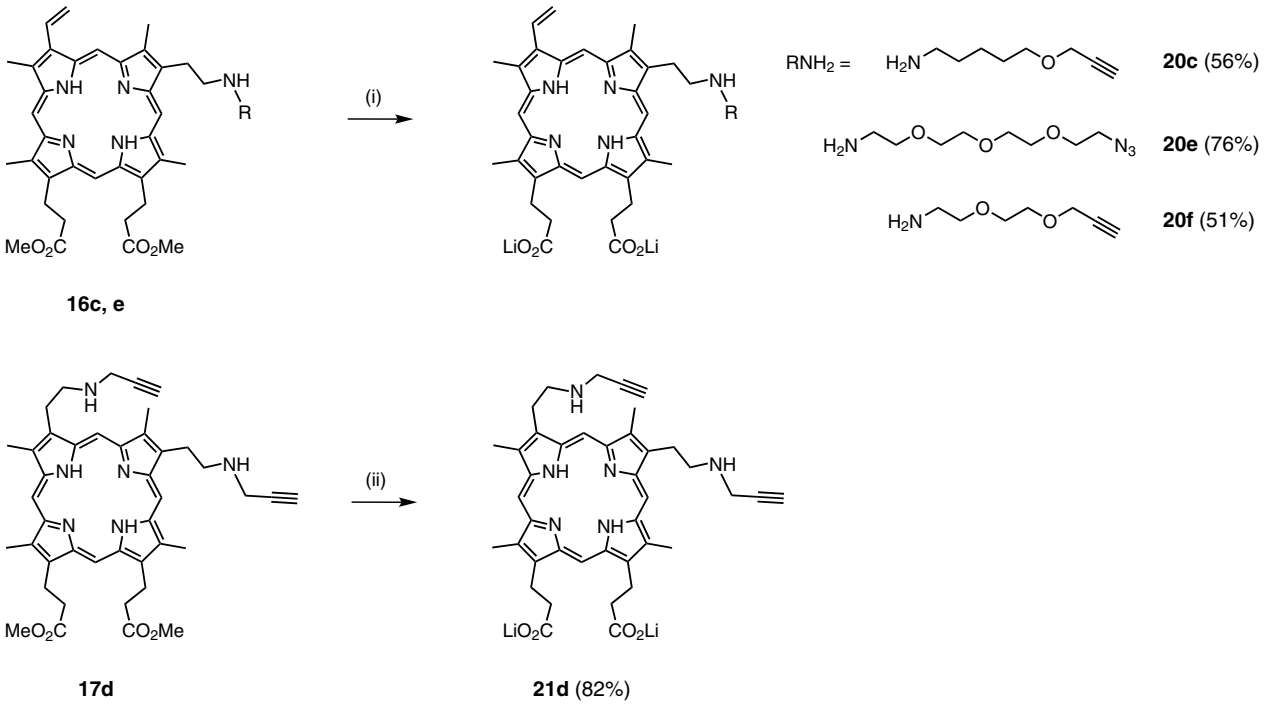


Scheme 10. Cycloaddition reaction of porphyrin derivative **16b** with vitamin alkyne derivative **18c**: (i) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, TBTA, *t*BuOH/H_2O (5:1), rt, 20 h

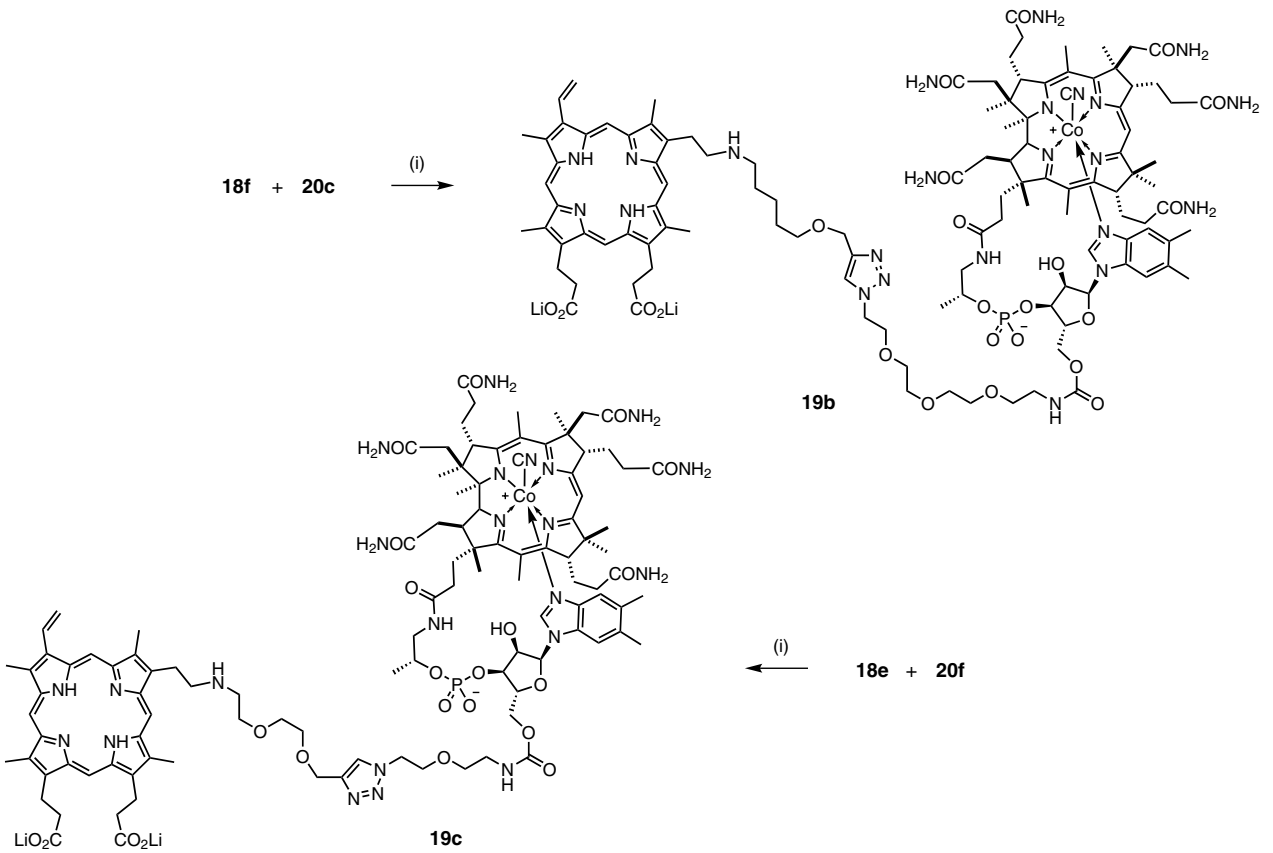
formation of the expected product **19a** was confirmed by MS (as a copper complex), but unfortunately hybrid **19a** was insoluble in any solvents, most importantly in water. It was probably due to the peculiar character of this molecule — it contains a strongly hydrophilic vitamin part and a hydrophobic porphyrin part.

Considering the potential application of the PPIX-vitamin B_{12} hybrids, their water solubility was crucial. Therefore, in the next reactions we decided to use PPIX derivatives with hydrolyzed ester groups. Hydrolysis of selected PPIX derivatives **16** and **17** was performed using NaOH in MeOH/H_2O or LiOH·H_2O in THF/H_2O/MeOH or dioxane/H_2O/MeOH (Scheme 11). When using NaOH, some side products of PPIX decomposition were formed and were very difficult to remove. Gratifyingly, hydrolysis with LiOH proceeded smoothly though purification and characterization of lithium salts **20** and **21** also presented difficulties. Reverse-phase C-18 silica gel could not be used because of too strong adsorption on stationary phase while cationites caused decomposition. A fairly good method for their purification turned out to be chromatography on lipophilic Sephadex with water and MeOH as eluents. Signals in NMR spectra of such amphiphilic porphyrins were broad, probably due to formation of micelle-like aggregates. The best results were obtained in DMSO-d_6 or sometimes in CD_3OD.

Water-soluble porphyrin lithium salts **20** and **21** were then employed in CuAAC reactions. In order to avoid copper insertion into the porphyrin core, we tried conditions different from those shown in Scheme 10, which was CuI/TBTA/sodium ascorbate in EtOH/H_2O 1:1 or in DMF. The reaction of azide **18f** with porphyrin alkyne **20c** in EtOH/H_2O led only to the recovery of the starting materials, while the reaction in DMF gave respective hybrids **19b and 19c** (Scheme 12). Under the developed conditions hybrid **19c** was obtained in good yield. These conjugates were fairly soluble in water and polar solvents like DMF or DMSO allowing their purification by precipitation and washing, similarly to vitamin derivatives **18**. However, chromatographic purification of these compounds was troublesome therefore their structure could only be, so far, confirmed by mass spectrometry. Importantly, in the case of compound **19b** ESI-MS spectrum demonstrated no incorporation of copper into the porphyrin core of the hybrid during the CuAAC reaction carried out with CuI/TBTA/sodium ascorbate catalytic system. Disappointingly, the main signal in the ESI-MS spectrum of hybrid **19c** was assigned as a copper complex. The isotopic distributions for the ESI-MS peaks of hybrids **19a**–**19c** matched the theoretical ones.



Scheme 11. Hydrolysis of the ester groups in PPIX derivatives **16**, **17**. (i) $LiOH \cdot H_2O$, dioxane/H_2O/MeOH; (ii) $LiOH \cdot H_2O$, THF/H_2O/MeOH



Scheme 12. Cycloaddition reactions. (i) CuI, TBTA, sodium ascorbate, DMF, rt

EXPERIMENTAL

General information

Analytical grade solvents were used as received. All reagents were purchased from Sigma Aldrich or POCH. ^{1}H and ^{13}C NMR spectra were recorded at rt on Bruker or Varian 500 MHz with TMS as an internal standard. DCVC (dry column vacuum chromatography) was performed using Merck Silica Gel (200–300 mesh). Analytical thin layer chromatography (TLC) was performed using Merck Silica Gel GF254, 0.20 mm thickness and preparative TLC on Merck Silica Gel 60, 1 mm thickness. Chromatographic purification of water-soluble compounds was performed of LiChroprep RP-18 gel or Lipophilic Sephadex LH-20. High resolution ESI mass spectra were recorded on a Mariner spectrometer. UV-vis absorption spectra were recorded in DCM on a Perkin Elmer λ-25.

Synthesis of PPIX derivatives with terminal azide and alkyne linkers

Oxidation of porphyrin (13) — preparation of PPIX adehydes 14 and 15 [14]. To a 250 mL round-bottom flask porphyrin **13** (0.542 mmol, 320 mg) and CH_2Cl_2 (120 mL) were added under an argon atmosphere. The mixture was heated to 43 °C and a solution of $Tl(NO_3)_3{\cdot}3H_2O$ (1.19 mmol, 530 mg) in MeOH (25 mL) was added in one portion with vigorous stirring of the reaction mixture. After 15 min (monitoring by TLC – 1% MeOH in CH_2Cl_2) the reaction mixture was cooled (cold water bath) and filtered through a pad of cotton. Conc. $HCl_{(aq)}$ (2 mL) was added to the filtrate and it was stirred vigorously. After about five minutes solid Na_2CO_3 and Na_2SO_4 were added and then the solution was filtered and evaporated. The residue (a mixture of dimethyl acetals of porphyrin) was dissolved in 85% formic acid (about 100 mL) and stored overnight at 0 °C or stirred at rt for 2 h. The acidic solution was then diluted with an equal amount of brine and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were washed with water (2 × 150 mL) and brine (150 mL), dried over a mixture of Na_2CO_3 (or K_2CO_3) and Na_2SO_4, filtrated and concentrated.

A mixture of PPIX monoaldehydes **14** was separated from bis(aldehyde) **15** by column chromatography on SiO_2 using 2–5% MeOH/CH_2Cl_2.

Monoaldehydes 14 [14]. Yield 40%. ^{1}H NMR (200 MHz; $CDCl_3$; Me_4Si): δ_H, ppm -3.84 (2H, bs), 3.27 (4H, *t*, $^3J_{HH}$ = 7.8 Hz), 3.45–3.65 (9H, m), 3.65–3.80 (9H, m), 4.38 (4H, m), 4.98 (2H, s), 6.18 (1H, d, $^3J_{HH}$ = 10.9 Hz), 6.36 (1H, d, $^3J_{HH}$ = 17.8 Hz), 8.25 (1H, dd, $^3J_{HH}$ = 17.1 Hz, 11.8 Hz), 9.80 (1H, s), 10.0 (2H, s), 10.1 (1H, s), 10.2 (1H, s).

Bis(aldehyde) 15 [14]. Yield 44%. ^{1}H NMR (200 MHz; $CDCl_3$; Me_4Si): δ_H, ppm -3.82 (2H, bs), 3.27 (4H, m), 3.57 (3H, s), 3.59 (6H, s), 3.61 (3H, s), 3.65 (6H, s), 4.39 (4H, t, $^3J_{HH}$ = 7.8 Hz), 5.02 (4H, s), 9.85 (2H, s), 10.0 (1H, s), 10.1 (1H, s), 10.2 (2H, s).

Reductive amination of PPIX aldehydes. A mixture of **14** and **15** (crude, obtained from 0.542 mmol of **13**) was dissolved in acetonitrile (75 mL) followed by the addition of acetic acid (250 µl) under an argon atmosphere. Then, over a 15–20 min time period a solution of primary amine (2.71 mmol) in MeCN (5 mL) and AcOH (250 µL) was added dropwise, simultaneously with portionwise addition of $NaBH_3CN$ (8.24 mmol, 511 mg). After 1 h of stirring, the reaction mixture was diluted with chloroform (c.a. 100 mL) and washed with water (3 × 100 mL) and saturated $Na_2CO_{3(aq)}$ (50 mL), dried over Na_2SO_4 and evaporated. The products were separated using chromatography on silica gel starting with 1-2% MeOH in CH_2Cl_2 up to 33% MeOH. Each product was then further purified on a new column or by using a preparative SiO_2 TLC plate.

Porphyrin 16a. Yield 80%. IR (KBr): ν_{max}, cm^{-1} 3311, 2924, 2857, 2688, 2091, 1733, 1434, 1361, 1194, 1162, 1105, 906, 833, 726, 676. HRMS (ESI): calcd. for $C_{42}H_{53}N_8O_4$ ([M + H]$^+$) 733.4184, found 733.4190. *1st isomer:* ^{1}H NMR (500 MHz; $CDCl_3$; Me_4Si): δ_H, ppm -4.62 (2H, bs), 0.86 (2H, m), 0.95–1.32 (6H, m), 2.91 (4H, m), 3.16 (4H, m), 3.40 (8H, m), 3.63 (3H, s), 3.64 (9H, s), 4.16–4.33 (6H, m), 6.02 (1H, dm, $^3J_{HH}$ = 10.5 Hz), 6.16 (1H, dm, $^3J_{HH}$ = 18 Hz), 7.96 (1H, dd, $^3J_{HH}$ = 17.2 Hz, 11.5 Hz), 9.33–9.80 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$; Me_4Si): δ_C, ppm 11.7, 11.4, 11.5, 12.5, 21.6, 21.7, 26.2, 26.4, 28.4, 29.6, 31.4, 31.9, 36.8, 36.8, 48.3, 48.4, 51.0, 51.7, 95.8, 96.1, 96.3, 96.7, 97.0, 97.1, 120.1, 120.2, 130.1, 136.5 (bm), 173.5, 173.5. *2nd isomer:* ^{1}H NMR (500 MHz, $CDCl_3$): δ_H, ppm -4.62 (2H, bs), 0.86 (2H, m), 0.95–1.32 (6H, m), 2.91 (4H, m), 3.16 (4H, m), 3.40 (8H, m), 3.63 (3H, s), 3.64 (9H, s), 4.16–4.33 (6H, m), 6.04 (1H, dm, $^3J_{HH}$ = 9.4 Hz), 6.20 (1H, dm, $^3J_{HH}$ = 18.4 Hz), 7.96 (1H, dd, $^3J_{HH}$ = 18.1 Hz, 12.0 Hz), 9.33–9.80 (4H, m). ^{13}C NMR (125 MHz; $CDCl_3$; Me_4Si): δ_C, ppm 11.7, 11.4, 11.6, 12.6, 21.6, 21.7, 26.2, 26.4, 28.5, 29.7, 31.4, 31.9, 36.8, 36.8, 48.3, 48.4, 51.0, 51.7, 95.8, 96.1, 96.3, 96.7, 97.0, 97.1, 120.1, 120.2, 130.1, 136.5 (bm), 173.5, 173.5.

Porphyrin 17a. Yield 15%. IR (KBr): ν_{max}, cm^{-1} 3445, 3311, 2934, 2858, 2326, 2092, 1732, 1435, 1362, 1259, 1195, 1164, 1105, 835, 726, 676. HRMS (ESI): calcd. for $C_{48}H_{67}N_{12}O_4$ ([M + H]$^+$) 875.5403, found 875.5394. ^{1}H NMR (600 MHz; $CDCl_3$; Me_4Si): δ_H, ppm -3.98 (2H, bs), 1.28–1.38 (8H, m), 1.48 (4H, m), 1.59 (4H, m), 2.60 (8H, m), 3.10 (8H, m), 3.25 (4H, m, $^3J_{HH}$ = 8.4 Hz), 3.46 (3H, s), 3.48 (3H, s), 3.54 (3H, s), 3.55 (3H, s), 3.64 (3H, s), 3.66 (3H, s), 3.98 (2H, t, $^3J_{HH}$ = 7.8 Hz), 4.05 (2H, t, $^3J_{HH}$ = 8.0 Hz), 4.34 (4H, m), 9.84 (1H, s), 9.90 (1H, s), 9.91 (1H, s), 9.99 (1H, s). ^{13}C NMR (150 MHz; $CDCl_3$; Me_4Si): δ_C, ppm 11.6, 11.6, 11.6, 21.8, 24.1, 24.2, 26.6, 26.7, 27.1, 27.1, 27.2, 27.2, 28.7, 28.8, 36.9, 42.4, 42.4, 51.3, 51.7, 51.7, 57.5, 57.6, 59.8, 59.9.

Porphyrin 16b. Yield 27%. IR (film): ν_{max}, cm^{-1} 3686, 3600, 3474, 2953, 2110, 1734, 1606, 1438, 1171, 841, 680. HRMS (ESI): calcd. for $C_{40}H_{49}N_8O_5$ ($[M + H]^+$) 721.3820, found 721.3856. UV-vis (CH_2Cl_2): λ_{max}, nm (log ε) 404 (5.16), 502 (3.98), 536 (3.95), 570 (3.87), 625 (3.49). *1st isomer:* ^1H NMR (500 MHz; $CDCl_3$; Me_4Si): δ_H, ppm -3.84 (2H, bs), 2.99 (2H, m), 3.11 (2H, m), 3.27 (4H, m), 3.45–3.54 (4H, m), 3.54–3.60 (6H, m), 3.61–3.64 (6H, m), 3.65–3.70 (8H, m), 4.21 (2H, m), 4.39 (4H, m), 6.16 (1H, dm, $^3J_{HH}$ = 11.6 Hz), 6.34 (1H, dm, $^3J_{HH}$ = 17.7 Hz), 8.26 (1H, dd, $^3J_{HH}$ = 17.8 Hz, 11.4 Hz), 9.99 (1H, s), 10.02 (1H, s), 10.06 (1H, s), 10.16 (1H, s). *2nd isomer:* ^1H NMR (500 MHz, $CDCl_3$): δ_H, ppm -3.84 (2H, bs), 2.99 (2H, m), 3.11 (2H, m), 3.27 (4H, m), 3.45–3.54 (4H, m), 3.54–3.60 (6H, m), 3.61–3.64 (6H, m), 3.65–3.70 (8H, m), 4.21 (2H, m), 4.39 (4H, m), 6.16 (1H, dm, $^3J_{HH}$ = 11.6 Hz), 6.34 (1H, dm, $^3J_{HH}$ = 17.7 Hz), 8.25 (1H, dd, $^3J_{HH}$ = 17.7 Hz, 11.7 Hz), 10.00 (1H, m), 10.03 (2H, s), 10.06 (1H, s), 10.12 (1H, s).

Porphyrin 16c. Yield 21%. HRMS (ESI): *m/z* calcd. for $C_{44}H_{57}N_8O_7$ ($[M + H]^+$) 809.4345, found 809.4326. ^1H NMR (500 MHz; $CDCl_3$; Me_4Si): δ_H, ppm -4.16 (2H, bs), 2.88 (2H, m), 2.94 (4H, m), 3.04 (2H, m), 3.10 (2H, m), 3.24 (4H, t, $^3J_{HH}$ = 7.6 Hz), 3.36 (4H, m), 3.42 (2H, m), 3.52 (3H, s), 3.55 (5H, m), 3.59 (3H, s), 3.65 (3H, s), 3.66 (3H, s), 3.67 (3H, s), 4.03 (2H, m), 4.34 (4H, m), 6.13 (1H, m), 6.28 (1H, d, $^3J_{HH}$ = 17.7 Hz, *1st isomer*), 6.31 (1H, d, $^3J_{HH}$ = *2nd isomer*), 8.15 (1H, dd, $^3J_{HH}$ = 18.0 Hz, 11.5 Hz, *1st isomer*), 8.19 (1H, dd, $^3J_{HH}$ = 17.8 Hz, 11.5 Hz, *2nd isomer*), 9.74–9.83 (2H, m), 9.87–10.05 (2H, m). *1st isomer:* ^{13}C NMR (125 MHz, $CDCl_3$; Me_4Si): δ_C, ppm 11.5, 11.5, 11.6, 11.6, 21.8, 36.9, 50.2, 51.5, 51.7, 69.4, 69.7, 69.9, 69.9, 70.0, 70.2, 96.0, 96.3, 97.0, 97.4, 120.4, 130.4, 136.2 (m), 137.4 (m), 173.5, 173.6. *2nd isomer:* ^{13}C NMR (125 MHz; $CDCl_3$; Me_4Si): δ_C, ppm 11.5, 11.6, 11.6, 11.8, 21.7, 36.8, 50.2, 51.4, 51.7, 69.4, 69.7, 69.9, 69.9, 70.1, 70.2, 96.0, 96.4, 96.9, 97.4, 120.4, 130.4, 136.2 (m), 137.4 (m), 173.5, 173.6. Anal. calcd. for $C_{44}H_{56}N_8O_7{\cdot}1.5H_2O$: C, 63.22; H, 7.11; N, 13.40%. Found: C, 63.15; H, 7.11; N, 13.20%. Anal. calcd. for $C_{44}H_{56}N_8O_7{\cdot}2H_2O$: C, 62.54; H, 7.16; N, 13.26%. Found: C, 62.63; H, 7.24; N, 13.42%.

Porphyrin 17c. Yield 5%. MS (ESI): *m/z* 514.4 ($[M + 2H]^{2+}$), 1027.7 ($[M + H]^+$). ^1H NMR (400 MHz; $CDCl_3$; Me_4Si): δ_H, ppm -3.78 (2H, bs), 3.02 (4H, m), 3.07, (8H, m), 3.14 (4H, m), 3.20 (4H, m), 3.29 (8H, m), 3.35–3.56 (12H, m), 3.67 (18H, m), 4.28 (4H, m), 4.43 (4H, m), 10.10 (2H, m), 10.12 (2H, m).

Porphyrin 16d. Yield 27%. IR (KBr): ν_{max}, cm^{-1} 3309, 2946, 2910, 1732, 1614, 1434, 1361, 1226, 1195, 1162, 1107, 905, 832, 725, 677. UV-vis (CH_2Cl_2): λ_{max}, nm (log ε) 331 (3.68), 404 (4.65), 502 (3.41), 537 (3.41), 570 (3.39), 602 (2.92), 626 (2.92). HRMS (ESI): calcd. for $C_{39}H_{44}N_5O_4$ ($[M + H]^+$) 646.3388, found 646.3383. ^1H NMR (500 MHz, DMSO-d_6, Me_4Si): δ_H, ppm -4.08 (2H, s), 3.11 (1H, m), ~3.30 (4H, m), 3.45 (2H, t, $^3J_{HH}$ = 7.6 Hz), 3.53–3.64 (20H, m), 3.67 (2H, s, *1st isomer*), 3.70 (2H, s, *2nd isomer*), 4.21 (2H, m), 4.28–4.42 (4H, m), 6.17 (1H, d, $^3J_{HH}$ = 11.5 Hz, *1st isomer*), 6.18 (1H, d, $^3J_{HH}$ = 11.6 Hz, *2nd isomer*), 6.39 (1H, d, $^3J_{HH}$ = 17.7 Hz, *1st isomer*), 6.41 (1H, d, $^3J_{HH}$ = 18.1 Hz, *2nd isomer*), 8.42 (1H, dd, $^3J_{HH}$ = 17.1 Hz, 11.6 Hz, *1st isomer*), 8.46 (1H, dd, $^3J_{HH}$ = 17.2 Hz, 11.6 Hz, *2nd isomer*), 10.11–10.17 (2H, m), 10.17–10.24 (2H, m). *1st isomer:* ^{13}C NMR (125 MHz; $CDCl_3$; Me_4Si): δ_C, ppm 11.6, 11.6, 11.7, 12.7, 21.7, 36.9, 37.9, 50.4, 51.7, 71.5, 81.8, 96.1, 96.4, 96.6, 96.9, 97.0, 97.5, 120.5, 130.4, 137 (m), 173.5, 173.6. *2nd isomer:* ^{13}C NMR (125 MHz; $CDCl_3$; Me_4Si): δ_C, ppm 11.6, 11.6, 11.8, 12.8, 21.8, 37.0, 37.9, 50.4, 51.7, 71.5, 81.8, 96.1, 96.4, 96.6, 96.9, 97.0, 97.5, 120.5, 130.4, 137 (m), 173.5, 173.6.

Porphyrin 17d. Yield 20%. IR (KBr): ν_{max}, cm^{-1} 3288, 2912, 2857, ~2130, 1732, 1637, 1435, 1363, 1226, 1195, 1163, 1108, 833, 737, 677. HRMS (ESI): calcd. for $C_{42}H_{49}N_6O_4$ ($[M + H]^+$) 701.3810, found 701.3831. ^1H NMR (400 MHz; $CDCl_3$; Me_4Si): δ_H, ppm -3.86 (2H, bs), 2.16 (2H, m), 3.28 (4H, t, $^3J_{HH}$ = 7.7 Hz), 3.56 (8H, m), 3.60 (3H, s), 3.63 (3H, s), 3.65 (6H, s), 3.66 (6H, s), 4.21 (4H, m), 4.41 (4H, t, $^3J_{HH}$ = 7.6 Hz), 10.03 (1H, s), 10.05 (2H, s), 10.07 (1H, s). ^{13}C NMR (100 MHz; $CDCl_3$; Me_4Si): δ_C, ppm 11.7, 11.8, 11.9, 21.8, 26.7, 36.9, 38.1, 50.6, 51.7, 51.7, 71.8, 81.6, 96.3, 96.7, 96.7, 96.8, 173.6. Anal. calcd. for $C_{42}H_{48}N_6O_4{\cdot}H_2O$: C, 70.17; H, 7.01; N, 11.69%. Found: C, 70.25; H, 6.78; N, 12.18%.

Porphyrin 16e. HRMS (ESI): calcd. for $C_{44}H_{54}N_5O_5$ ($[M + H]^+$) 732.4119, found 732.4123. ^1H NMR (600 MHz; $CDCl_3$; Me_4Si): δ_H, ppm -5.07 (2H, bs), 1.38 (2H, m), 1.50 (2H, m), 1.86 (2H, m), 2.29 (1H, t, $^4J_{HH}$ = 2.2 Hz), 2.89 (2H, m), 3.05 (2H, m), 3.11 (2H, m), 3.27 (2H, m), 3.33 (2H, m), 3.49 (3H, s), 3.52 (3H, s), 3.59 (3H, s), 3.63 (3H, s), 3.63 (3H, s), 3.64 (3H, s), 3.93 (2H, m), 4.11 (2H, m), 4.15 (4H, m), 5.94 (1H, m), 6.06 (1H, d, $^3J_{HH}$ = 18 Hz, *1st isomer*), 6.11 (1H, d, $^3J_{HH}$ = 18 Hz, *2nd isomer*), 7.77 (1H, dd, $^3J_{HH}$ = 18 Hz, 12 Hz, *1st isomer*), 7.86 (1H, dd, $^3J_{HH}$ = 18 Hz, 12 Hz, *2nd isomer*), 9.23 (1H, s), 9.39–9.64 (3H, m).

Porphyrin 17e. Yield 4%. HRMS (ESI): calcd. for $C_{52}H_{69}N_6O_6$ ($[M + H]^+$) 873.5273, found 873.5301.

Porphyrin 16f. Yield 25%. HRMS (ESI): calcd. for $C_{43}H_{52}N_5O_6$ ($[M + H]^+$) 734.3912, found 734.3909. ^1H NMR (500 MHz; $CDCl_3$; Me_4Si): δ_H, ppm -4.16 (2H, bs), 2.16 (1H, m), 2.96 (2H, m), 3.23 (6H, m, $^3J_{HH}$ = 7.4 Hz), 3.41 (6H, m), 3.42 (5H, m), 3.55–3.63 (7H, m), 3.66 (6H, m), 3.73 (2H, m), 4.02–4.17 (2H, m), 4.32 (4H, m), 6.13 (1H, dm, $^3J_{HH}$ = 11.3 Hz), 6.29 (1H, dm, $^3J_{HH}$ = 17.7 Hz), 8.16 (1H, m, $^3J_{HH}$ = 17.2 Hz, 11.5 Hz), 9.80 (1H, s), 9.90 (2H, s), 10.00 (1H, s). ^{13}C NMR (125 MHz; $CDCl_3$; Me_4Si): δ_C, ppm 11.5, 11.6, 11.6, 11.8, 12.7, 12.8, 21.7, 21.8, 36.8, 36.9, 36.9, 48.5, 48.6, 51.1, 51.2, 51.7, 58.0, 58.0, 68.6, 68.6, 69.1 (m), 70.0, 70.1, 74.4, 74.4, 79.2, 96.0, 96.3, 96.5, 96.9, 97.0, 97.4, 120.4, 130.4, 136.0 (m), 137.4 (m), 173.5, 173.6.

Porphyrin 17f. Yield 5%. HRMS (ESI): calcd. for $C_{50}H_{65}N_6O_8$ ([M + H]$^+$) 877.4858, found 877.4860.

Porphyrin 16g. Yield 30%. IR (KBr): ν_{max}, cm^{-1} 3309, 2912, 2862, 2446, 2111, 1732, 1610, 1435, 1349, 1228, 1195, 1164, 1104, 912, 832, 726, 676. HRMS (ESI): calcd. for $C_{45}H_{56}N_5O_7$ ([M + H]$^+$) 778.4174, found 778.4166. ^{1}H NMR (500 MHz; $CDCl_3$; Me_4Si): δ_H, ppm -4.37 (2H, s), 2.26 (1H, m, $^4J_{HH}$ = 2.2 Hz), 2.90 (2H, m), 3.00 3.09 (4H, m), 3.12 (2H, m), 3.19 (4H, m), 3.340–3.42 (6H, m), 3.44 (3H, s), 3.45 (3H, s), 3.52 (3H, s), 3.63 (3H, s), 3.64 (3H, s), 3.66 (3H, s), 3.84 (1H, d, $^4J_{HH}$ = 2.1 Hz, *1st isomer*), 3.86 (1H, d, $^4J_{HH}$ = 2.1 Hz, *2nd isomer*), 3.98 (2H, t, $^3J_{HH}$ = 7.2 Hz, *1st isomer*), 4.05 (2H, t, $^3J_{HH}$ = 7.3 Hz, *2nd isomer*), 4.23 (4H, m), 6.06 (1H, d, $^3J_{HH}$ = 11.8 Hz, *1st isomer*), 6.09 (1H, d, $^3J_{HH}$ = 11.7 Hz, *2nd isomer*), 6.21 (1H, d, $^3J_{HH}$ = 17.8 Hz, *1st isomer*), 6.26 (1H, d, $^3J_{HH}$ = 17.9 Hz, *2nd isomer*), 8.03 (1H, dd, $^3J_{HH}$ = 17.9 Hz, 11.6 Hz, *1st isomer*), 8.11 (1H, dd, $^3J_{HH}$ = 17.7 Hz, 11.5 Hz, *2nd isomer*), 9.64–9.90 (4H, m). ^{13}C NMR (125 MHz; $CDCl_3$; Me_4Si): δ_C, ppm 11.1, 11.1, 11.2, 11.5, 12.5, 12.9, 36.3, 36.3, 46.4, 48.6, 51.3, 57.3, 57.4, 65.9, 68.4, 69.4, 69.6, 69.7, 69.8, 77.1, 80.2, 96.6, 96.9, 97.0, 97.1, 97.2, 97.2, 120.4. 130.0, 137 (m), 172.9, 172.9, 172.9. UV-vis (CH_2Cl_2): λ_{max}, nm (log ε) 403 (5.03), 502 (3.91), 536 (3.83), 571 (3.74), 625 (3.41).

Porphyrin 17g. Yield 9%. IR (KBr): ν_{max}, cm^{-1} 3444, 3261, 2945, 2866, 2447, 2111, 1733, 1438, 1351, 1251, 1196, 1104, 836, 732. HRMS (ESI): calcd. for $C_{54}H_{73}N_6O_{10}$ ([M + H]$^+$) 965.5383, found 965.5382. UV-vis (CH_2Cl_2): λ_{max}, nm (log ε) 402 (5.03), 498 (3.77), 533 (3.77), 567 (3.77, 621 (3.27).

Hydrolysis of PPIX derivatives. Dimethyl ester of a porphyrin derivative (0.130 mmol) was dissolved in dioxane or THF (9 mL) and MeOH (1.5 mL). LiOH·H_2O (3.25 mmol, 136 mg) and water (3 mL) were added and the reaction mixture was stirred under an argon atmosphere for 24 h. The reaction mixture was diluted with water (15 mL) and extracted with CH_2Cl_2 (3 × 10 mL). Water phase was diluted with a few volumes of EtOH and evaporated. The residue was dissolved in water (2-3 mL), separated on a lipophilic sephadex column using first water and then 30% MeOH in water and evaporated.

Porphyrin 20c. Yield 56%. HRMS (ESI): *m/z* calcd. for $C_{42}H_{50}N_5O_5$ ([M + 3H]$^+$) 704.3806, found 704.3802.

Porphyrin 20e. Yield 76%. HRMS (ESI): *m/z* calcd. for $C_{42}H_{53}N_8O_7$ ([M + 3H]$^+$) 781.4032, found 781.4040. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H, ppm -3.92 (2H, bs), 2.88 (4H, m), 3.00–3.90 (34H, m), 4.28 (6H, m), 6.18 (1H, d, $^3J_{HH}$ = 11.2 Hz), 6.42 (1H, d, $^3J_{HH}$ = 17.6 Hz), 8.49 (1H, m), 10.19 (1H, s), 10.28 (2H, s), 10.85 (1H, s).

Porphyrin 21d. Yield 82%. HRMS (ESI): *m/z* calcd. for $C_{40}H_{45}N_6O_4$ ([M + 3H]$^+$) 673.3497, found 673.3509. ^{1}H NMR (500 MHz, CD_3OD): δ_H, ppm 3.14 (2H, m), 3.16 (2H, m, $^4J_{HH}$ = 1.6 Hz), 3.30 (6H, m), 3.39 (2H, m), 3.53 (5H, m), 3.59 (3H, s), 3.67 (m, 6H), 4.04 (2H, m), 4.16 (2H, m), 4.44 (4H, m), 9.93 (1H, s), 10.07 (1H, s), 10.09 (1H, s), 10.34 (1H, s).

Preparation of vitamin B_{12} derivatives with azide and alkyne linkers at 5' position

Vitamin B_{12} (0.15 mmol, 200 mg) was dissolved in dry, degassed DMSO (5 mL, ultrasonic bath, bubbling argon for 15 min) at 35 °C under an argon atmosphere. With stirring, solid CDT (0.225 mmol, 37 mg) was added. After 30 min, amine (0.5 mmol) was added in dry DMSO (0.5 mL). After 30 min the heating bath was removed and the reaction mixture was stirred overnight. The reaction mixture was then added dropwise to a vigorously stirred 1:1 mixture of Et_2O and CH_2Cl_2 (50 mL). After 30 min the clear solution above the red precipitate was removed by decantation, the precipitate was separated using a centrifuge, washed twice with CH_2Cl_2/Et_2O 2:1 and three times with Et_2O. After drying in air, the precipitate was dissolved in a small amount of water and separated using chromatography on RP gel using 0–30% EtOH in H_2O. After evaporation, the product was dissolved in DMF (2 mL) and further purified using the same precipitation, centrifugation and washing procedure as described above.

Vitamin 18a. Yield 35%. HRMS (ESI): *m/z* calcd. for $C_{67}H_{91}N_{15}O_{15}PCoNa_2$ ([M + 2Na]$^{2+}$) 740.7837, found 740.7808. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H, ppm 0.34 (3H, s), 0.85 (1H, m), 1.01 (3H, s), 1.03 (3H, s), 1.20 (3H, s), 1.26 (3H, s), 1.35 (3H, s), ~1.4 (1H, m), 1.55 (1H, m), 1.71 (3H, s), 1.73–1.80 (5H, m), 2.05 (3H, m), 2.19 (3H, s), 2.21 (3H, s), 2.15–2.35 (5H, m), 2.42 (3H, s), 2.35–2.51 (5H, m), 2.50 (3H, s), 2.70 (1H, m), 2.80 (1H, m), 2.92 (1H, m), 3.11 (1H, m), 3.17–3.25 (2H, m), 3.66 (1H, m), 3.81 (2H, m), 3.96 (1H, m), 4.08 (1H, m), 4.10–4.20 (3H, m), 4.33 (1H, d, $^3J_{HH}$ = 10.2 Hz), 4.65 (1H, m), 4.69 (1H, d, $^3J_{HH}$ = 8.3 Hz), 5.92 (1H, s), 6.24 (1H, s), 6.43 (1H, s), 6.46 (1H, s), 6.57 (1H, s), 6.80 (1H, s), 6.83 (1H, s), 7.03 (1H, s), 7.07 (1H, s), 7.10 (1H, s), 7.19 (1H, s), 7.24 (1H, s), 7.37 (1H, s), 7.41 (1H, d, J_{HH} = 7.2 Hz), 7.58 (1H, s), 7.62 (1H, s), 7.73 (1H, s), 7.75 (1H, s), 7.80 (1H, s).

Vitamin 18b. Yield 82%. IR (KBr): ν_{max}, cm^{-1} 3346, 3200, 2936, 2134, 1668, 1573, 1498, 1402, 1222, 1144, 1083, 559. HRMS (ESI): *m/z* calcd. for $C_{73}H_{103}N_{15}O_{18}PCoNa_2$ ([M + 2Na]$^{2+}$) 806.8230, found 806.8231. ^{1}H NMR (600 MHz, D_2O): δ_H, ppm 0.30 (3H, s), 0.88 (1H, m), 0.95 (1H, m), 1.05 (3H, s), 1.11 (3H, d, $^3J_{HH}$ = 6.5 Hz), 1.24 (3H, s), 1.26 (3H, s), 1.30 (3H, s), 1.66–1.72 (2H, m), 1.72 (3H, s), 1.75–1.85 (4H, m), 1.85 (2H, m), 1.98 (1H, td, $^3J_{HH}$ = 14.1 Hz, 3.2 Hz), 2.04 (1H, d, $^3J_{HH}$ = 13.7 Hz), 2.12 (6H, s), 2.26 (2H, AB, $^2J_{HH}$ = 13.3 Hz), 2.30–2.57 (7H, m), 2.40 (3H, s), 2.42 (3H, s), 2.61 (2H, m), 2.86 (1H, m), 3.15–3.24 (3H, m), 3.29 (1H, dd, $^3J_{HH}$ = 10.9, 5.2 Hz), 3.44–3.49 (5H, m), 3.49–3.56 (6H, m), 3.94 (1H, d, $^3J_{HH}$ = 10.1 Hz), 4.01 (2H, s), 4.04 (1H, dd, $^3J_{HH}$ = 8.9 Hz, 2.0 Hz), 4.07 (1H, m), 4.15 (1H, m), 4.18 (1H, m), 4.46 (1H, d, $^3J_{HH}$ = 11.3 Hz), 4.60–4.71 (2H, m), 5.93 (1H, s), 6.18 (1H, d, $^3J_{HH}$ = 2.4 Hz), 6.36 (1H, s), 6.96 (1H, s), 7.13 (1H, s). ^{13}C NMR (150 MHz, D_2O): δ_C, ppm 15.0, 15.2, 15.6, 16.6, 18.8,

18.8, 19.1, 19.1, 19.2, 19.7, 25.8, 27.7, 31.1, 31.2, 31.5, 31.7, 31.9, 34.2, 34.7, 36.8, 38.8, 40.1, 42.5, 42.7, 44.9, 47.0, 47.9, 51.2, 53.4, 55.5, 56.1, 57.7, 58.9, 62.7, 68.5, 68.5, 69.2, 69.4. 69.4, 69.5, 72.8, 73.1, 74.7, 78.8, 78.9, 79.7, 84.9, 86.8, 94.6, 103.9, 107.4, 111.2, 116.3, 129.7, 133.0, 135.0, 136.4, 141.6, 158.0, 165.1, 165.8, 173.4, 174.4, 174.9, 175.4, 175.6, 176.7, 176.8, 177.5, 177.6, 178.8, 179.9. UV-vis (H_2O): λ_{max}, nm (log ε) 278 (4.13), 305 (3.91), 322 (3.83), 361 (4.39), 408 (3.49), 548 (3.88), 968 (1.92).

Vitamin 18c. Yield 69%. HRMS (ESI): *m/z* calcd. for $C_{73}H_{103}N_{15}O_{16}Na_2PCo$ ($[M + 2Na]^{2+}$) 790.8280, found 790.8242. ^{1}H NMR (600 MHz, DMSO-d_6): δ_H, ppm 0.32 (3H, s), 0.86 (1H, m), 0.99 (3H, s), 1.02 (3H, s), 1.06 (3H, s), 1.18 (3H, s), 1.21–1.32 (4H, m), 1.33 (3H, s), 1.35–1.41 (2H, m), 1.45–1.52 (3H, m), 1.60–1.74 (2H, m), 1.69 (3H, s), 1.75–1.84 (4H, m), 1.90–2.10 (3H, m), 2.12–2.26 (4H, m), 2.16 (3H, s), 2.18 (3H, s), 2.27–2.45 (7H, m), 2.30 (3H, s), 2.39 (2H, t, $^3J_{HH}$ = 5.4 Hz), 2.48 (3H, s), 2.57 (1H, m), 2.77 (1H, m), 2.89 (1H, m), 2.95 (2H, m), 3.09 (1H, d, $^3J_{HH}$ = 10.7 Hz), 3.15 (1H, dd, $^3J_{HH}$ = 10.5 Hz, 5.6 Hz), 3.25 (1H, dd, $^3J_{HH}$ = 10.5 Hz, 6.0 Hz), 3.41 (2H, m), 3.69 (1H, m), 3.91 (1H, d, $^3J_{HH}$ = 10.8 Hz), 4.01 (1H, m), 4.06 (1H, m), 4.09 (1H, dm, $^4J_{HH}$ = 2.4 Hz), 4.15 (1H, m), 4.29 (1H, d, $^3J_{HH}$ = 9.8 Hz), 4.61 (1H, m), 4.69 (1H, d, $^3J_{HH}$ = 7.0 Hz), 5.90 (1H, s), 6.28 (1H, s), 6.36 (1H, s), 6.44 (1H, s), 6.53 (1H, s), 6.74 (1H, s), 6.78 (1H, s), 7.03 (1H, s), 7.07 (1H, s), 7.08 (1H, s), 7.13 (1H, s), 7.18 (1H, s), 7.36 (2H, s), 7.54 (1H, s), 7.68 (1H, s), 7.71 (1H, s), 7.78 (1H, s), 7.81 (1H, s). ^{13}C NMR (150 MHz, DMSO-d_6): δ_C, ppm 15.0, 15.2, 16.4, 16.5, 18.7, 19.8, 19.9, 20.0, 20.1, 20.1, 25.3, 25.4, 26.0, 26.1, 27.1, 28.7, 28.8, 29.3, 29.9, 31.0, 31.6, 31.7, 34.0, 35.1, 38.0, 40.2, 42.0, 42.1, 44.6, 46.5, 47.3, 50.3, 52.9, 54.0, 54.9, 57.2, 58.6, 62.8, 67.2, 68.8, 69.1, 70.3, 74.8, 76.8, 76.9, 80.6, 84.4, 85.9, 93.5, 103.1, 105.8, 111.8, 116.4, 129.7, 131.3, 132.6, 136.2, 142.2, 156.1, 164.6, 165.4, 170.9, 171.1, 172.5, 172.8, 173.2, 173.3, 173.5, 173.6, 174.0, 178.2, 179.5.

Vitamin 18d. Yield 63%. IR (KBr): ν_{max}, cm^{-1} 3334, 3195, 2934, 2097, 1668, 1573, 1498, 1401, 1238, 1145, 1071, 559. HRMS (ESI): *m/z* calcd. for $C_{70}H_{100}N_{18}O_{15}PCoNa$ ($[M + Na]^+$) 1545.6577, found 1545.6519. ^{1}H NMR (600 MHz, DMSO-d_6): δ_H, ppm 0.29 (3H, s), 0.84 (1H, bs), 1.01 (3H, d, $^3J_{HH}$ = 6.6 Hz), 1.02 (3H, s), 1.14 (3H, s), 1.20 (3H, s), 1.25 (4H, m), 1.29 (3H, s), 1.37 (2H, m), 1.47 (2H, m), 1.56–1.70 (2H, m), 1.66 (3H, s), 1.70–1.80 (5H, m), 1.95–2.40 (3H, m), 2.13 (3H, s), 2.14 (3H, s), 2.15–2.28 (3H, m), 2.33 (1H, m), 2.36 (3H, s), 2.38–2.50 (6H, m), 2.44 (3H, s), 2.55 (1H, m), 2.74 (1H, m), 2.84 (1H, m), 2.92 (2H, m), 3.06 (1H, d, $^3J_{HH}$ = 10.7 Hz), 3.27 (2H, t, $^3J_{HH}$ = 7.1 Hz), 3.42 (1H, m), 3.65 (1H, dd, $^3J_{HH}$ = 9.3, 5.4 Hz), 3.91 (1H, d, $^3J_{HH}$ = 10.5 Hz), 3.96 (1H, d, $^3J_{HH}$ = 10.1 Hz), 4.03 (2H, m), 4.11 (1H, m), 4.25 (1H, d, $^3J_{HH}$ = 10.1 Hz), 4.66 (2H, m), 5.84 (1H, s), 6.31 (1H, d, $^3J_{HH}$ = 9.1 Hz), 6.40 (1H, s), 6.50 (1H, s), 6.75 (2H, s), 6.97 (1H, s), 6.99 (1H, s), 7.05 (1H, s), 7.09 (1H, s), 7.14 (1H, s), 7.32 (1H, s), 7.44 (1H, s), 7.50 (2H, s), 7.67 (2H, s), 7.76 (1H, s), 7.80 (1H, s). ^{13}C NMR (150 MHz, DMSO-d_6): δ_C, ppm 15.4, 16.1, 16.8, 16.9, 19.1, 20.2, 20.3, 20.4, 20.5, 20.5, 26.0, 26.1, 26.3, 27.6, 28.6, 29.7, 30.3, 31.5, 31.9, 32.1, 32.1, 34.3, 35.6, 38.5, 40.6, 42.1, 42.6, 45.0, 47.0, 47.8, 50.7, 51.0, 53.4, 54.4, 55.4, 59.0, 63.4, 69.3, 70.7, 73.3, 75.2, 79.9, 84.8, 86.5, 94.0, 103.5, 106.3, 112.2, 116.7, 130.2, 131.6, 132.9, 136.7, 142.6, 156.6, 165.0, 165.8, 171.4, 171.5, 172.9, 173.0, 173.2, 173.7, 174.0, 174.3, 175.5, 178.7, 179.9. UV-vis (H_2O): λ_{max}, nm (log ε) 278 (4.15), 305 (3.92), 322 (3.84), 361 (4.41), 408 (3.49), 519 (3.83), 549 (3.89), 962 (0.48).

Vitamin 18e. Yield 38%. ^{1}H NMR (600 MHz, DMSO-d_6): δ_H, ppm 0.29 (3H, s), 0.84 (1H, m), 1.02 (3H, s), 1.02 (3H, s), 1.14 (3H, s), 1.20 (3H, s), 1.29 (3H, s), 1.46 (1H, m), 1.61 (1H, m), 1.66 (3H, s), 1.67–1.80 (5H, m), 2.00 (3H, m), 2.13 (3H, s), 2.14 (3H, s), 2.15–2.21 (3H, m), 2.25 (1H, m), 2.32 (1H, m), 2.35 (3H, s), 2.38–2.51 (5H, m), 2.44 (3H, s), 2.57 (1H, m), 2.75 (1H, m), 2.86 (1H, m), 3.05 (1H, d, $^3J_{HH}$ = 10.4 Hz), 3.11 (2H, m), 3.30–3.42 (5H, m), 3.54 (2H, m), 3.65 (1H, m), 3.91 (1H, d, $^3J_{HH}$ = 10.5 Hz), 3.98 (1H, d, $^3J_{HH}$ = 9.9 Hz), 4.03 (2H, m), 4.13 (1H, m), 4.26 (1H, d, $^3J_{HH}$ = 10.0 Hz), 4.66 (2H, m), 5.84 (1H, s), 6.27 (1H, s), 6.30 (1H, s), 6.40 (1H, s), 6.50 (1H, s), 6.76 (2H, s), 6.97 (1H, s), 7.00 (1H, s), 7.05 (1H, s), 7.09 (1H, s), 7.13 (1H, s), 7.33 (1H, s), 7.42 (1H, s), 7.50 (1H, s), 7.65 (1H, s), 7.72 (2H, s), 7.83 (1H, s). ^{13}C NMR (150 MHz, DMSO-d_6): δ_C, ppm 15.5, 16.1, 16.8, 16.9, 19.1, 20.2, 20.3, 20.3, 20.5, 20.5, 26.0, 26.1, 27.5, 30.3, 31.5, 31.9, 32.1, 32.1, 34.3, 35.6, 38.5, 40.5, 42.1, 42.5, 45.0, 47.0, 47.8, 50.4, 50.7, 53.4, 54.4, 55.4, 59.0, 63.6, 69.3, 69.4, 69.4, 70.8, 73.2, 75.2, 79.9, 84.8, 86.5, 94.0, 103.5, 106.3, 112.1, 116.7, 130.2, 131.6, 133.0, 136.7, 142.6, 156.7, 165.0, 165.8, 171.4, 171.6, 173.0, 173.0, 173.2, 173.7, 174.0, 174.4, 175.5, 178.7, 179.9. UV-vis (H_2O): λ_{max}, nm (log ε) 279 (4.17), 306 (3.94), 323 (3.89), 361 (4.41), 518 (3.86), 548 (3.92).

Vitamin 18f. Yield 63%. IR (KBr): ν_{max}, cm^{-1} 3347, 3197, 2934, 2109, 1669, 1573, 1498, 1402, 1224, 1144, 1082, 559. HRMS (ESI): *m/z* calcd. for $C_{72}H_{104}N_{18}O_{18}PCoNa_2$ ($[M + 2Na]^{2+}$) 822.3315, found 822.3296. ^{1}H NMR (600 MHz, DMSO-d_6): δ_H, ppm 0.29 (3H, s), 0.84 (1H, m), 1.01 (3H, d, $^3J_{HH}$ = 6.2 Hz), 1.02 (3H, s), 1.14 (3H, s), 1.20 (3H, s), 1.30 (3H, s), 1.46 (1H, m), 1.58–1.71 (2H, m), 1.66 (3H, s), 1.76 (4H, m), 2.01 (3H, m), 2.13 (3H, s), 2.15 (3H, s), 2.14–2.27 (3H, m), 2.33 (2H, m), 2.35 (3H, s), 2.38–2.48 (3H, m), 2.44 (3H, s), 2.51 (2H, s), 2.55 (1H, m), 2.75 (1H, m), 2.86 (1H, m), 3.06 (1H, d, $^3J_{HH}$ = 11.0 Hz), 3.09 (2H, m, $^3J_{HH}$ = 5.7 Hz), 3.35 (2H, t, $^3J_{HH}$ = 4.8 Hz), 3.38 (2H, t, $^3J_{HH}$ = 6.2 Hz), 3.42 (1H, m), 3.45–3.51 (6H, m), 3.51–3.53 (2H, m), 3.56 (2H, t, $^3J_{HH}$ = 5.0 Hz), 3.65 (1H, dd, $^3J_{HH}$ = 9.5, 5.5 Hz), 3.91 (1H, d, $^3J_{HH}$ = 10.7 Hz), 3.98 (1H, m), 4.01 (1H, m), 4.05 (1H, m), 4.12 (1H, m), 4.23 (1H, d, $^3J_{HH}$ = 10.0 Hz), 4.66 (2H, m), 5.84 (1H, s), 6.31 (2H, bs), 6.40 (1H, s), 6.49 (1H, s), 6.75 (2H, s), 6.95 (1H, s), 6.99 (1H, s),

7.06 (1H, s), 7.09 (1H, s), 7.14 (1H, s), 7.32 (1H, s), 7.42 (1H, s), 7.50 (1H, s), 7.65 (1H, s), 7.68 (1H, s), 7.76 (1H, s), 7.81 (1H, s). ^{13}C NMR (150 MHz, DMSO-d_6): δ_C, ppm 15.5, 16.1, 16.8, 16.9, 19.1, 20.2, 20.3, 20.4, 20.5, 20.5, 26.0, 26.1, 27.5, 30.3, 31.5, 31.9, 32.1, 32.1, 34.4, 35.6, 38.5, 40.9, 42.1, 42.5, 45.0, 47.0, 47.8, 50.4, 50.7, 53.4, 54.4, 55.4, 59.0, 63.6, 69.3, 69.6, 69.7, 70.0, 70.1, 70.2, 70.2, 70.7, 73.2, 75.2, 79.9, 84.8, 86.5, 94.0, 103.5, 106.3, 112.2, 116.7, 130.2, 131.6, 133.0, 136.7, 142.7, 156.7, 165.0, 165.8, 171.4, 171.5, 172.9, 173.0, 173.2, 173.7, 174.0, 174.3, 175.5, 178.7, 179.9. UV-vis (H_2O): λ_{max}, nm (log ε) 278 (4.15), 305 (3.93), 322 (3.85), 361 (4.40), 408 (3.52), 519 (3.84), 549 (3.89), 962 (1.59).

CuAAC reaction of alkine 18c with azide 16b in *t*-BuOH/H_2O – hybrid 19a

PPIX azide derivative **16b** (12 μmol, 8.9 mg), TBTA (16 μmol, 8.3 mg), sodium ascorbate (78 μmol, 15.5 mg) and vitamin B_{12} alkyne derivative **18c** (13 μmol, 20.0 mg) were added to degassed (ultrasonic bath, bubbling Ar for 15 min) mixture of water and *tert*-BuOH (1:3.7, 2.5 mL). After stirring for 10 min $CuSO_4 \cdot 5H_2O$ (16 μmol, 4.0 mg) was added and the reaction mixture was stirred at rt for 20 h. By TLC analysis, the starting PPIX derivative and most of vitamin B_{12} derivative was consumed. A dark red precipitate was formed. It was filtered, washed with water (3 × 2 mL), *tert*-BuOH (2 mL) and CH_2Cl_2 (3 × 2 mL). The solid obtained after drying (6 mg) was insoluble in any common solvents (DMF, DMSO, etc.). It was analyzed using MS ESI spectroscopy.

Hybrid 19a. MS (ESI): *m/z* 2319.8 ($[C_{113}H_{150}N_{23}O_{21}PCoCu]^+$),1159.9($[C_{113}H_{151}N_{23}O_{21}PCoCu]^{2+}$).

CuAAC reaction in DMF

CuI (80 μmol, 15 mg) and TBTA (201 μmol, 107 mg) were dissolved to dry, degassed (ultrasonic bath, bubbling Ar for 15 min) DMF (5 mL). After 15 min, when the solution was completely clear, vitamin derivative (**18f** or **18e**, 30.7 μmol), PPIX derivatived (**20c** or **20f**, 28 μmol) and sodium ascorbate (160 μmol, 32 mg) were added. The reaction mixture was stirred overnight at rt and then it was added dropwise to a vigorously stirred mixture of Et_2O and CH_2Cl_2 (1:1, 25 mL). After 30 min the clear solution above the red precipitate was removed by decantation, the precipitate was separated using a centrifuge, washed twice with CH_2Cl_2/Et_2O 2:1 and three times with Et_2O. After drying, the precipitate was dissolved in water and separated by chromatography on RP gel. Unreacted vitamin B_{12} was first removed with 10% EtOH in H_2O, and the new product was then eluted with 50% EtOH in H_2O or 50% *i*-PrOH in H_2O, in some difficult cases basified with 2% LiOH. After evaporation it was dissolved in DMF (1.5–2 mL), precipitated and washed in the same way as described for the crude reaction mixture.

Hybrid 19b. MS (ESI): *m/z* 1147.9 ($[C_{113}H_{151}N_{22}O_{23}PCoLi_3]^{2+}$).

Hybrid 19c. MS (ESI): *m/z* 1140.5 ($[C_{109}H_{143}N_{23}O_{22}PCoCu]^{2+}$).

CONCLUSION

A series of PPIX derivatives containing one or two linkers, attached through the PPIX vinyl group making them suitable for CuAAC reaction, was synthesized. Considering the versatility of CuAAC, these derivatives could be used for bioconjugation with a broad variety of substrates. We have also showed that propionyl methyl esters could be easily hydrolyzed with lithium hydroxide in good yield. CuAAC reactions of porphyrin derivatives with suitably functionalized vitamin B_{12} derivatives gave desired, water soluble hybrids. The presence of free carboxylate groups in PPIX part are the prerequisite for hybrid's water solubility. Interestingly, under the optimal conditions for CuAAC copper insertion into the PPIX part of the hybrid was avoided, which is a rare case in the chemistry of porphyrins. Further effort is needed to develop methods of processing and purification of such hybrids to enable their appropriate characterization and pharmacological evaluation.

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Supporting information

Experimental procedures for the synthesis of linkers **6** and **10** 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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