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Vitamin B_{12} Derivatives for Orthogonal Functionalization

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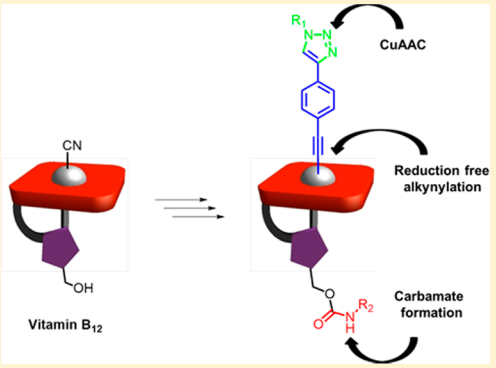
Supporting Information

ABSTRACT: The synthesis of vitamin B_{12} derivatives for selective orthogonal conjugation at both the Co center and 5′-OH is reported. Newly developed, reduction-free, direct alkynylation of vitamin B_{12} at the central cobalt ion proved to be versatile, with the formed acetylides, unlike other metalloorganic derivatives, showing remarkable heat and light stability, thus making them promising candidates as a drug carrier. Subsequently, high-yielding functionalization can be achieved via a sequence of selective [1,3] dipolar azide−alkyne cycloadditions (AACs) or carbamate formation followed by AAC.



INTRODUCTION

In mammalian cells vitamin B_{12} (cobalamin, Cbl; Figure 1) is vital for the proper functioning of two enzymes: methionine synthase and methylmalonyl-CoA mutase.[1−3] This exogenous molecule is provided from food, and a unique uptake system enables its delivery to cells.[1,3] The transport process relies on vitamin B_{12} being recognizable and bound by a sequence of transport proteins present in the gastrointestinal system: transcobalamin I (TCI) in the mouth, intrinsic factor (IF) in the stomach, and transcobalamin II (TCII) in the intestines.[1−5] This established delivery pathway[4,5] makes vitamin B_{12} an attractive candidate as a drug carrier—an auxiliary moiety attached to therapeutics facilitating their transportation to the place of action.[6,7] In order to utilize this unique system, vitamin B_{12} must be properly modified and then conjugated to an active drug molecule. Such modifications affect the recognition of vitamin B_{12} by glycoproteins, but this strongly depends on the selected conjugation site.[6] Wilbur examined the influence of vitamin B_{12} functionalization on binding to TCII.[8] When positions *b* and *e* were used as attachment sites, conjugates were recognized by the glycoprotein; similar effects were observed for modifications at the central Co ion and at the 5′-OH group (Figure 1). However, functionalization at position *c* or *d* led to a dramatic drop in binding properties. These experimental data, confirmed later by an X-ray structure of the B_{12}-TCII complex,[9] have paved the way for future developments of vitamin B_{12} chemistry over the last few years.

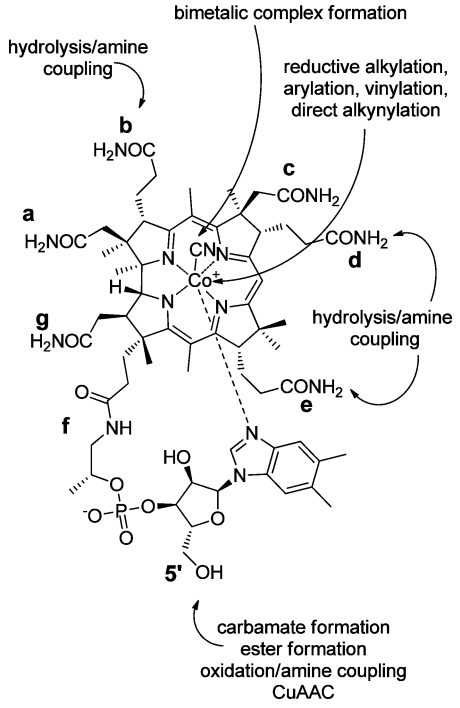


Figure 1. Structure of cyanocobalamin, group labeling, and conjugation sites.

The complexity of the Cbl molecule makes selective functionalizations at certain positions very difficult, while conjugates with two moieties attached are unprecedented. There are several methods for cobalamin modification, with only a few being selective.[10] Carefully controlled partial hydrolysis of cyanocobalamin under acidic conditions gives access to desirable *e* and *b* acids, which can only be accessed via a sequence of complex chromatographic purifications; accordingly, the final yields are very low.[8,11]

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The earliest methods for 5′-OH functionalization relied on the reaction of cyanocobalamin ((CN)Cbl) with anhydrides, furnishing unstable esters.[12] By far the most commonly utilized method for the purpose of conjugation is the carbamate or carbonate methodology introduced by Russell-Jones.[13,14] The hydroxyl group at position 5′ is first reacted with a carbonyl group equivalent—1,1′-carbonyldiimidazole (CDI) or 1,1′-carbonylbis(1,2,4-triazole) (CDT)—and then treated with an amine. This gave way to the preparation of vitamin B_{12} conjugates with various compounds of confirmed biological activity, especially peptide therapeutics[7,15,16] such as insulin[17] and appetite-suppressing peptide drugs,[18] as well as functionalization of therapeutic nucleic acids[15] and fluorescent probes.[19,20] In spite of the apparent advantages, some drawbacks, such as partial conversion and moderate yields, are present. Furthermore, CDT-activated vitamin B_{12} reacts with any nucleophilic group present in a substrate structure; thus, the whole procedure often requires additional protection/deprotection steps or laborious purifications. Alternatively, the 5′-OH group can be oxidized to the corresponding carboxylic acid using the 2-iodoxybenzoic acid (IBX)/2-hydroxypyridine (HYP) system as an oxidant and then coupled with amines.[21] A new and effective approach, developed recently by our group, relied on [1,3] dipolar cycloaddition.[22] The 5′-OH was transformed into a good leaving group and subsequently substituted with an azide. The resulting "clickable" azide is stable and highly active in the copper-catalyzed as well as in the strain-promoted [1,3] dipolar cycloaddition (CuAAC or SPAAC) to alkynes.

Functionalization at the cobalt ion can be accomplished by either alkylation[10] or utilization of cyanide ligand properties to act as an electron pair donor for transition metals, resulting in bimetallic complexes.[8,23] The synthesis of organometallic species requires reduction of the cobalt(III) to cobalt(I) cobalamin and its subsequent reaction with electrophiles: alkyl halides, acyl halides, Michael acceptors, epoxides, etc.[10] Reduced B_{12} Cbl(II) was found to react with an aryl diazonium salt, furnishing arylcobalamin presumably via a radical reaction.[24] Most metalloorganic derivatives are highly unstable: they are heat and light sensitive and thus are difficult to handle.[25] Nevertheless, using this methodology Grissom et al. prepared colchicine-B_{12} hybrid and showed that the derivative was toxic to cancer cells, expressed higher selectivity toward tumors, and showed fewer side effects than the parent drug.[26] Alberto's group conjugated (CN)Cbl with platinium-based anticancer drugs by linking them to the CN^- ligand; the Co-CN-Pt motif formed.[27,28] The resulting hybrids showed excellent affinity for all sets of B_{12} transport proteins, comparable with that of vitamin B_{12}.

Recently, three groups independently described the reaction of vitamin B_{12} with phenylacetylene derivatives leading to organometallic derivatives. Marques found that the reaction of Cbl(I) with phenylacetylene furnished unstable β-phenylvinyl cobalamin.[29] Two other groups reported the synthesis of remarkably stable acetylide cobalamins. When reduced vitamin B_{12} was reacted with 2-phenylethynyl iodide, Cbl(II) intermediate was trapped by 2-phenylethynyl radical, leading to Co−C_{sp} bond formation.[30] In parallel, our group established that the reduction step was not required; direct reaction of (CN)Cbl with terminal alkynes in the presence of Cu(I) salts furnished acetylides in excellent yields.[31] To the best of our knowledge, this was the first example of an efficient, operationally simple (no anhydrous and anaerobic conditions required) method for the preparation of organometallic vitamin B_{12} derivatives without the need for troublesome cobalt reduction. Our preliminary data strongly suggested that this method is not limited to arylacetylenes but can be extended to other terminal alkynes.[31]

Herein, we present a detailed study on acetylide cobalamins, examining their unique properties in relation to the Co−C bond and their utility in the synthesis of selectively, orthogonally functionalized vitamin B_{12} derivatives, unheard of conjugates with two moieties attached.

RESULTS AND DISCUSSION

Alkynylation of Vitamin B_{12}. The reduction-free method for alkynylation of (CN)Cbl was found to be efficient for a small set of useful phenylacetylene derivatives.[31] To fully recognize its potential, scope and limitation studies were performed. Phenylacetylenes bearing electron-donating and electron-withdrawing groups at different positions were reacted with cobalamin under previously established conditions: (CN)Cbl (0.5 M), CuOAc (1.0 equiv), phenylacetylene (10 equiv), and DBU (1.5 equiv) in DMA at room temperature for 3 h, furnishing the desired products in decent yields (Table 1).

Cbl-phenylacetylides **1** bearing electron-withdrawing groups were obtained in decent yields (Table 1, entries 1−4). In order to sidestep possible dimer formation or Glaser-type couplings,[32] the synthesis of acetylide **1g** was performed at higher dilution (entry 7), and the progress of the reaction was closely monitored as the selectivity was decreasing over time (Glaser coupling products detected by MS). In the case of phenylacetylenes with electron-donating groups the situation became more complex (entries 8−11); 4-CH_2OH- and 4-OMe-phenylacetylenes afforded high yields of expected products (entries 8 and 9), while the use of highly electron rich 2-ethynyl-1,3,5-trimethoxybenzene furnished a complex mixture of unstable compounds (entry 10). 4-Ethynylaniline and its Boc-protected derivative (entries 11 and 12) gave only traces of desired products. Furthermore, aliphatic alkynes bearing functional groups suitable for further functionalization were tested (Figure 2).

Reactions with propargyl alcohols worked well, giving the expected products **2a**,**b** in 52% and 53% yields, respectively. When the hydroxyl group was transformed into an aryl ether (**2d**), the yield increased appreciably to 91% (Figure 2). An even more pronounced difference was observed for reactions with propiolic acid and the respective ester; only the latter allowed the preparation of acetylide **2c** in 85% yield.[31] Even though the list of acetylenes studied is not exhaustive, our alkynylation method proved to be quite general.

The lengths of the Co−C bonds in acetylide cobalamins are much shorter than those in other types of organometallic B_{12} derivatives (by approximately 0.1 Å in comparison to methylcobalamin) but fluctuate depending on the electronic nature of the substituent.[31] Despite such variations in structural features, all of these compounds show remarkable heat and light stability, unlike other metalloorganic B_{12} derivatives. pH stability tests were performed for the respective compounds **1a**,**f**, **i** and **2a** by measuring a series of absorption spectra in buffered solutions within the pH range from 1 to 8. The pH value at which the hypsochromic shift of all characteristic bands (γ at 363 nm, β at 522 nm, and α at 550 nm) occurred strongly depended on the nature of the substituent (Figure 3).

Such changes in absorption are attributed to an exchange of the acetylide ligand with H_2O leading to aquacobalamin.[30] For

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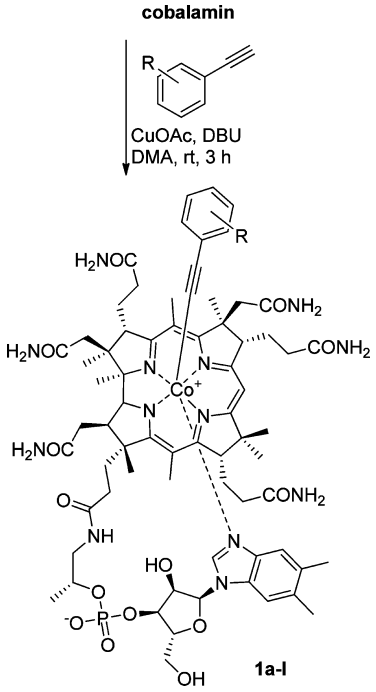
Table 1. Reduction-Free Alkynylation of Vitamin B_{12}: Scope and Limitations[a]



entry	R	product	yield (%)[b]
1	4-NO_2	**1a**	90[c]
2	4-CN	**1b**	64[d]
3	4-CF_3	**1c**	70
4	2-CF_3	**1d**	81
5	4-Br	**1e**	71
6	H	**1f**	85[c]
7	4-HC≡C	**1g**	70[e]
8	4-CH_2OH	**1h**	91
9	4-OMe	**1i**	80[c]
10	2,4,6-$(OMe)_3$	**1j**	0[f]
11	4-NH_2	**1k**	traces[g]
12	4-NHBoc	**1l**	traces[g]

[a]Reaction conditions: (CN)Cbl (0.10 mmol, 136 mg), CuOAc (0.10 mmol, 12 mg), and DBU (0.15 mmol, 22 μL) were dissolved in DMA (2 mL) and mixed at room temperature for 3 h. [b]Isolated yields. [c]See ref 31. [d]Unidentified byproducts observed (HPLC). [e]Performed on a 0.30 mmol scale; reaction time 2.5 h. [f]Complex reaction mixture; three main products were unstable and thus not isolated. [g]Very low conversion observed (HPLC).

compound **1i**, bearing a strongly electron donating 4-OMe substituent, the process occurred at pH 5−4, while for phenylacetylides **1f** and **2a**, it occurred at pH 2−3. Compound **1a** was the most resistant toward acidic hydrolysis, since no substantial changes in spectrum appeared until pH 1.

We hypothesized that this remarkable stability of acetylides is due to the nature of the Co−C bond. Thus, we have performed DFT calculations to gain some information on the bonding interaction between these two centers (Tables 2 and 3). Expectedly, the calculated charges confirm that the Co−C bond is polarized, though less than in cyanocobalamin (Table 2). The comparison of calculated and experimental (X-ray data)[31] bond lengths are in good agreement and enabled us to perform an analysis of the Co−C bond. The WBI index value for a typical single bond should be <1, and for acetylide derivatives it is ∼0.8.[33]

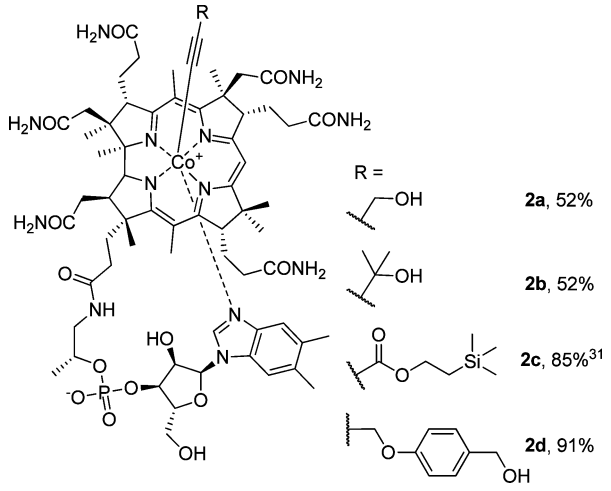


Figure 2. Aliphatic acetylides **2**.

Functionalization of Vitamin B_{12} using an Alkynylation/AAC Sequence. We have previously reported the synthesis of a "clickable" vitamin B_{12} derived azide and showed that it was active in [1,3] dipolar azide−alkyne cycloaddition (AAC).[22,34] However, only with our new method in hand it was possible to obtain the complementary vitamin B_{12} derivative **1g**, possessing an orthogonal handle in the form of a terminal triple bond. Consequently, we tested whether it was reactive in [1,3] dipolar azide−alkyne cycloaddition. Acetylide **1g** was subjected to the reaction with benzyl azide in the presence of CuI/TBTA (tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine) (Scheme 1).[35] The desired compound **3a** was isolated in 75% yield, with only traces of unidentified byproducts being observed. Another azide, namely 2-(2-azidoethoxy)ethanol, worked equally well, affording conjugate **3b** in satisfactory yield (78%).

This data suggested that the sequential, selective functionalization of cyanocobalamin at two positions, the central cobalt atom and the 5′-position, would be feasible. Two approaches were considered: (1) the use of two consecutive AAC reactions and (2) the carbamate ligation/CuAAC sequence.

The first approach required introduction of two "clickable" groups into the vitamin B_{12} structure. Using methodologies recently developed in our group,[22] we decided to incorporate both "clickable" groups, azide and the terminal triple bond, into a single molecule and to attempt two subsequent AACs on vitamin B_{12}. Thus, mesylate **4** was alkynylated with 1,4-diethynylbenzene and subsequent nucleophilic substitution gave the azide/alkyne-functionalized B_{12} derivative **6** in 95% isolated yield (65% overall yield based on vitamin B_{12}) (Scheme 2).[22]

With the "doubly clickable" derivative **6** in hand, we decided to conjugate it by an SPAAC/CuAAC sequence (Scheme 3). Since SPAAC is driven only by the high reactivity of strained octyne and does not require an external stimulus (catalyst, elevated temperature, etc.), possible side reactions leading to dimers etc. were thus ruled out.[36,37] Additionally, in most cases SPAAC proceeds quantitatively; hence, assuming full conversion of starting material **6**, a one-pot SPAAC/CuAAC sequence was envisaged. Azide **6** was reacted with *endo*-9-hydroxymethylbicyclo[6.1.0]nonyne (**7**)[38] for 18 h, and then CuI/TBTA and BnN_3 were added. HPLC analysis of the crude reaction mixture revealed that the SPAAC step was indeed nearly quantitative, and the following CuAAC furnished

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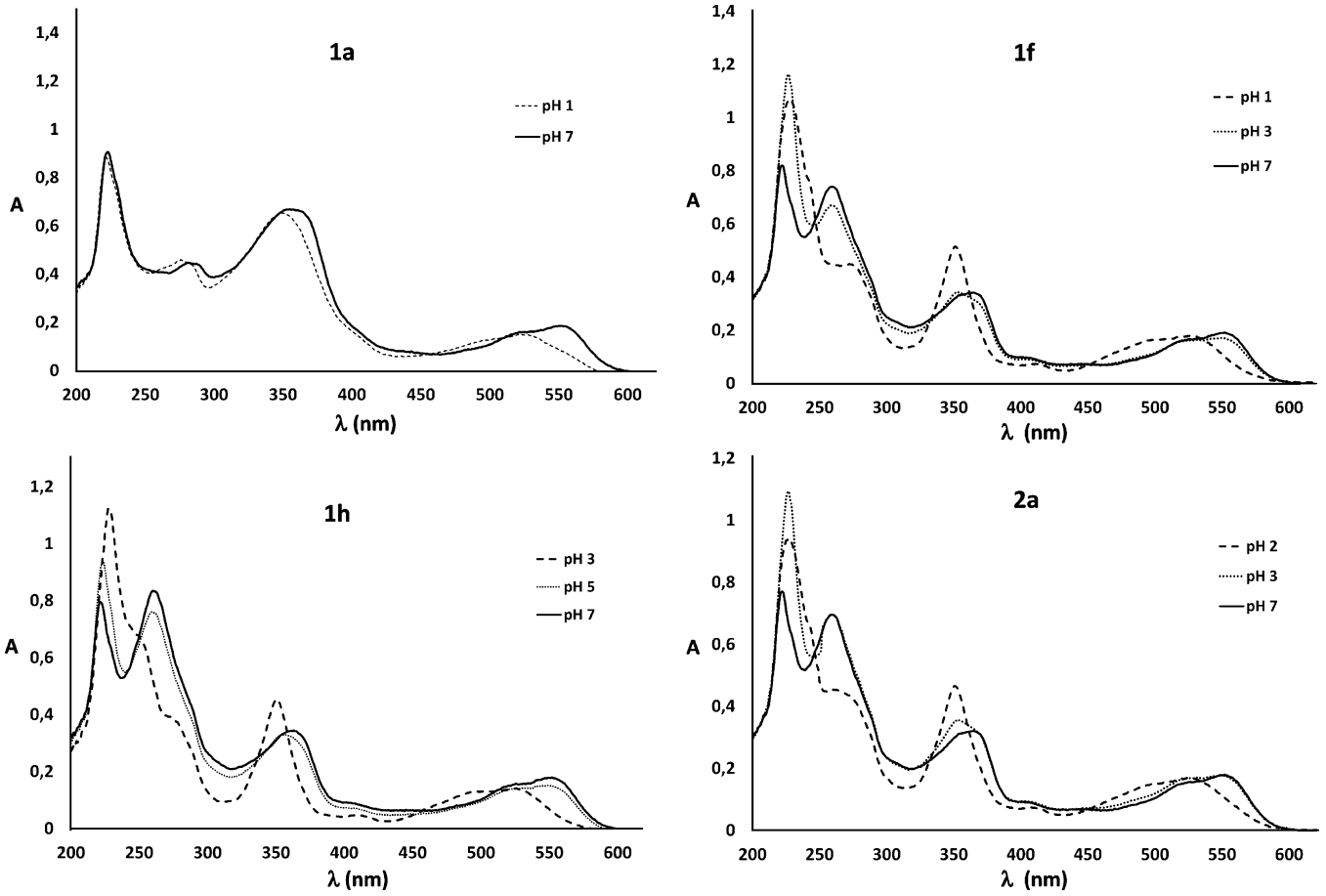

Figure 3. pH dependence of UV/vis spectra of compounds **1a**,**f**,**h** and **2a**.

Table 2. Comparison of Wiberg Bond Indices (WBI) Values and the Calculated Co−C Distances for Different Ligands[a]

		NBO charge		WBI		
entry	ligand	C	Co	Co−β-C	Co−α-N	Co−C distance (Å)[b]
1	CN−	0.21638	0.17527	0.8154	0.1062	1.875[c]
2	HC≡C−	−0.07118	0.24894	0.8297	0.3717	1.881
3	PhC≡C−	−0.01451	0.25241	0.8255	0.3757	1.883 (1.870)

[a]Level of theory: B3LYP/6-31G(d) for C, H, N, O, and P and LANL2DZ for Co. [b]Experimental values are given in parentheses. [c]See ref 1.

Table 3. NBO Analysis for Co−C Bonds[a]

entry	substituent	bond polarization (%)	*s* character (%)	*p* character (%)	*d* character (%)
1	CN−	34.33 (Co)	22.00	48.08	29.92
		65.67 (C)	54.57	45.42	0.01
2	HC≡C−	37.67 (Co)	35.93	16.18	47.89
		62.33 (C)	47.56	52.43	0.01
3	PhC≡C−	38.64 (Co)	28.92	16.83	54.25
		61.36 (C)	47.93	52.06	0.01

[a]Level of theory: B3LYP/6-31G(d) for C, H, N, O, and P and LANL2DZ for Co.

difunctionalized cobalamin **8** in 84% isolated yield as a mixture of two regioisomers.

Furthermore, the reaction of acetylide **6** with benzyl azide catalyzed by CuI/TBTA afforded desired the cycloadduct **9** in 81% yield within 18 h with no dimer being formed (Scheme 3). Hence, we decided to perform two consecutive CuAACs as a one-pot procedure.

Azide **6** was first reacted with benzyl azide in the presence of CuI/TBTA, and after full conversion of the starting material was achieved (HPLC analysis after overnight reaction), phenylacetylene was added to the reaction mixture (an excess of phenylacetylene was used to react with the remaining azide). After 24 h the final product **10** was isolated in 64% yield (approximately 80% per CuAAC step). The opposite reactions sequence, e.g. the reaction of **6** with phenylacetylene followed by addition of benzyl azide, gave similar results (60% yield, according to HPLC analysis of the crude reaction mixture). This situation clearly highlighted the versatility of our methodology in the selective orthogonal difunctionalization of vitamin B_{12}.

Alternatively, CuAAC for functionalization of the acetylide moiety could be combined with the well-established Russell-Jones carbamate methodology for conjugation at 5′-OH (Scheme 4).[14]

The reaction of acetylide **1g** with CDT and then with dipeptide **11** afforded the desired derivative **12** in 92% yield.[39] Subsequent cycloaddition with D-biotin-6-azido-*n*-hexylamide

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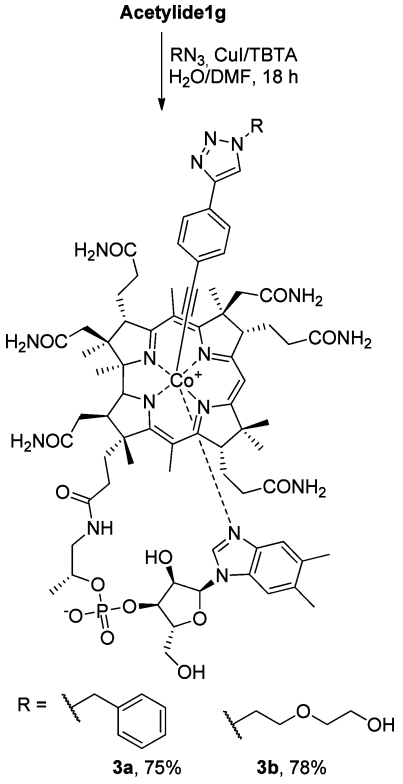
Scheme 1. Syntehsis of Alkyne 1g and Its Application for the CuAAC Reaction



(**13**) catalyzed by CuI/TBTA furnished difunctionalized conjugate **14** in 51% overall yield after four steps (based on vitamin B_{12}).

Alknylation of vitamin B_{12} opened a new avenue, leading to orthogonally functionalized derivatives, which proved a valuable starting material for further modifications by either an amidation/AAC sequence or by two consequtive AACs.

CONCLUSIONS

In summary, direct, reduction-free alkynylation of vitamin B_{12} proved a suitable method for the synthesis of both phenyl- and alkylacetylides. Using this methodology "doubly clickable" vitamin B_{12}, a valuable building block for further functionalization via [1,3] dipolar azide−alkyne cycloaddition, was prepared. A combination of AAC (CuAAC and SPAAC) with the carbamate method opened a new synthetic route to orthogonally difunctionalized vitamin B_{12} derivatives conjugated at the central Co ion and the 5′-position.

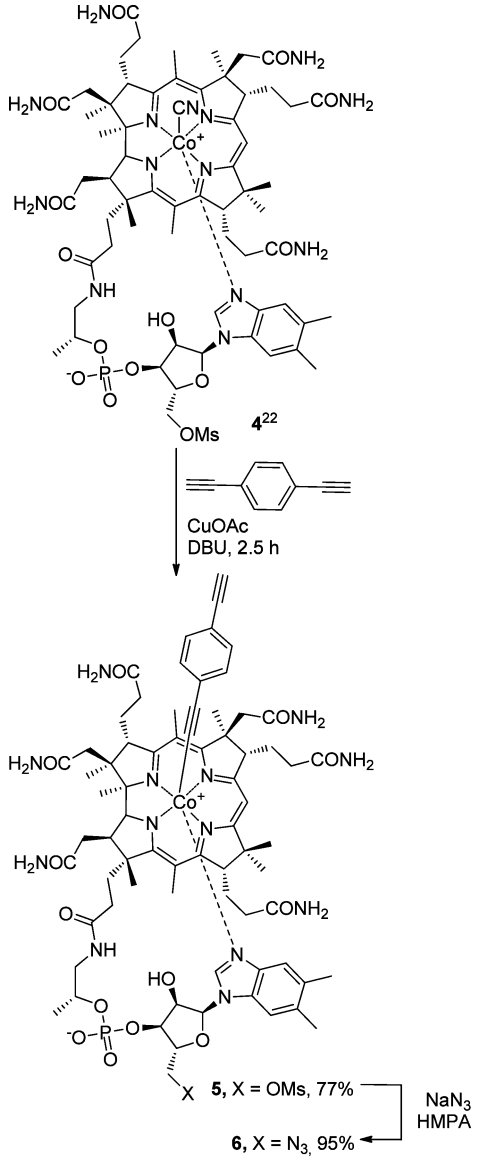
EXPERIMENTAL SECTION

General Information. Vitamin B_{12}, other reagents, and solvents were used as received. Preparative chromatography was performed using silica gel 90 C18 and redistilled water and HPLC grade MeCN as eluents. NMR spectra were recorded at room temperature on 600 and 500 MHz spectrometers with the residual solvent peak as an internal standard. All compounds cocrystallized with either organic solvent or water, which could not be removed by heating to 50 °C under vacuum. HRMS spectra were recorded on a spectrometer with TOF mass analyzer. All reactions and product purities were monitored using HPLC techniques.

Compounds **1a,f,i**, and **2c**[31] and **4**[22] were obtained as previously described.

HPLC measurement conditions: column, Eurospher II 100-5, C18, 250 mm × 4.6 mm with a precolumn; detection, UV/vis, wavelength λ

Scheme 2. Synthesis of Compound 6



361 nm; flow rate, 1 mL/min; pressure, 10 MPa; temperature, 30 °C; solvent A, aqueous 0.05% TFA; solvent B, MeCN.

The HPLC method is given in Table 4.

General Procedure for Synthesis of Compounds 1a−l and 2a−d. Vitamin B_{12} (136 mg, 0.10 mmol) and CuOAc (12 mg, 0.1 mmol) were dissolved in DMA (2.0 mL) followed by the addition of the proper alkyne (1.0 mmol). To the vigorously stirred solution was added DBU (22 μL, 0.2 mmol), and the reaction mixture was stirred at room temperature for 3 h. The crude product was precipitated with Et_2O (15 mL) and centrifuged. The resulting precipitate was washed once with AcOEt (15 mL) and twice with Et_2O (2 × 15 mL), followed by centrifugation and drying in air. The resulting solid was then dissolved in distilled water (20 mL) and centrifuged, and the solution over the yellowish precipitate was collected and concentrated in vacuo. The red solid was charged on an RP C18 column and eluted with H_2O/MeCN (gradually from 10 to 70% MeCN). Fractions containing the desired product were collected and concentrated in vacuo. The obtained red solid was dissolved in MeOH (2 mL), precipitated with Et_2O (15 mL), centrifuged, and dried overnight under reduced pressure at 50 °C.

*Acetylide **1b**:* yield 93 mg (64%); red powder, mp >225 °C dec; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.69 (s, 1H), 7.58 (s, 1H), 7.56 (s, 1H), 7.54 (s, 2H), 7.51 (s, 2H), 7.33 (s, 1H), 7.30 (s, 1H), 7.16 (s,

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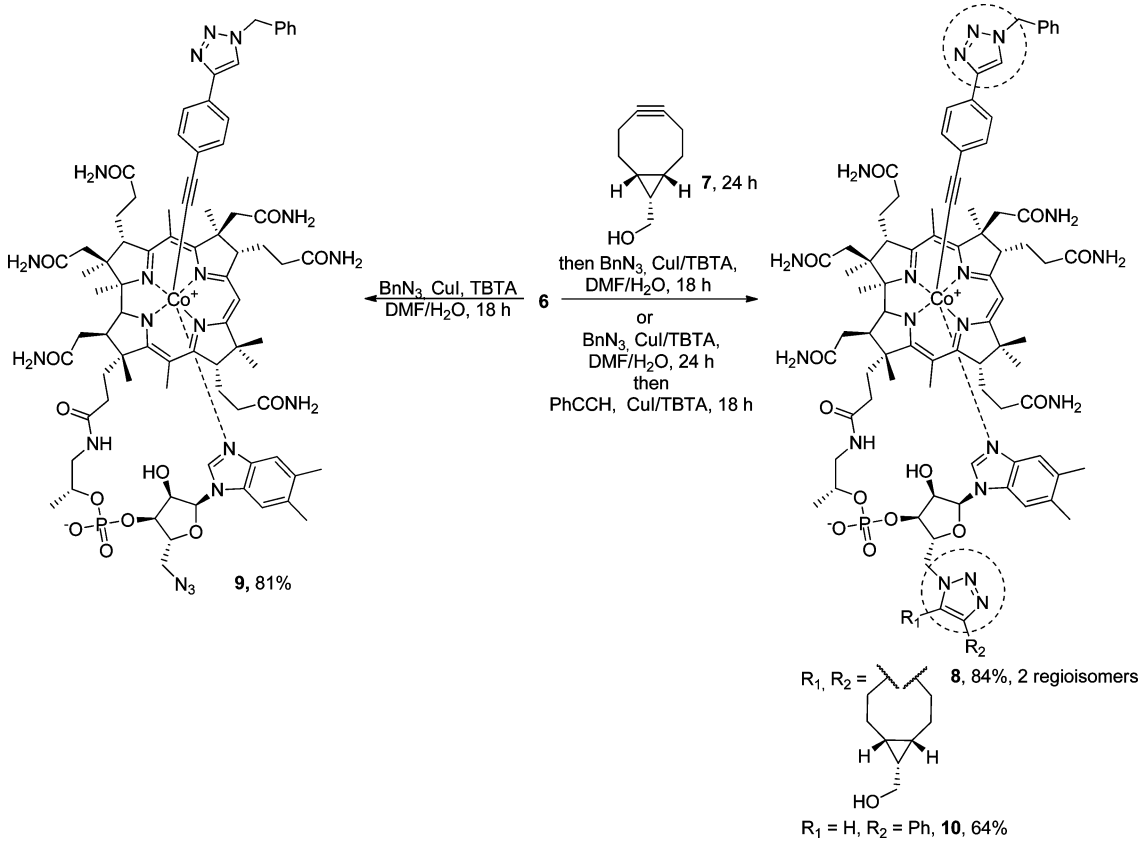
Scheme 3. Application of Compound 6 for CuAAC Reactions: One-Pot SPAAC/CuAAC Sequence on Compound 6



Scheme 4. Functionalization of Compound 1g with Peptide and D-Biotin Derivative

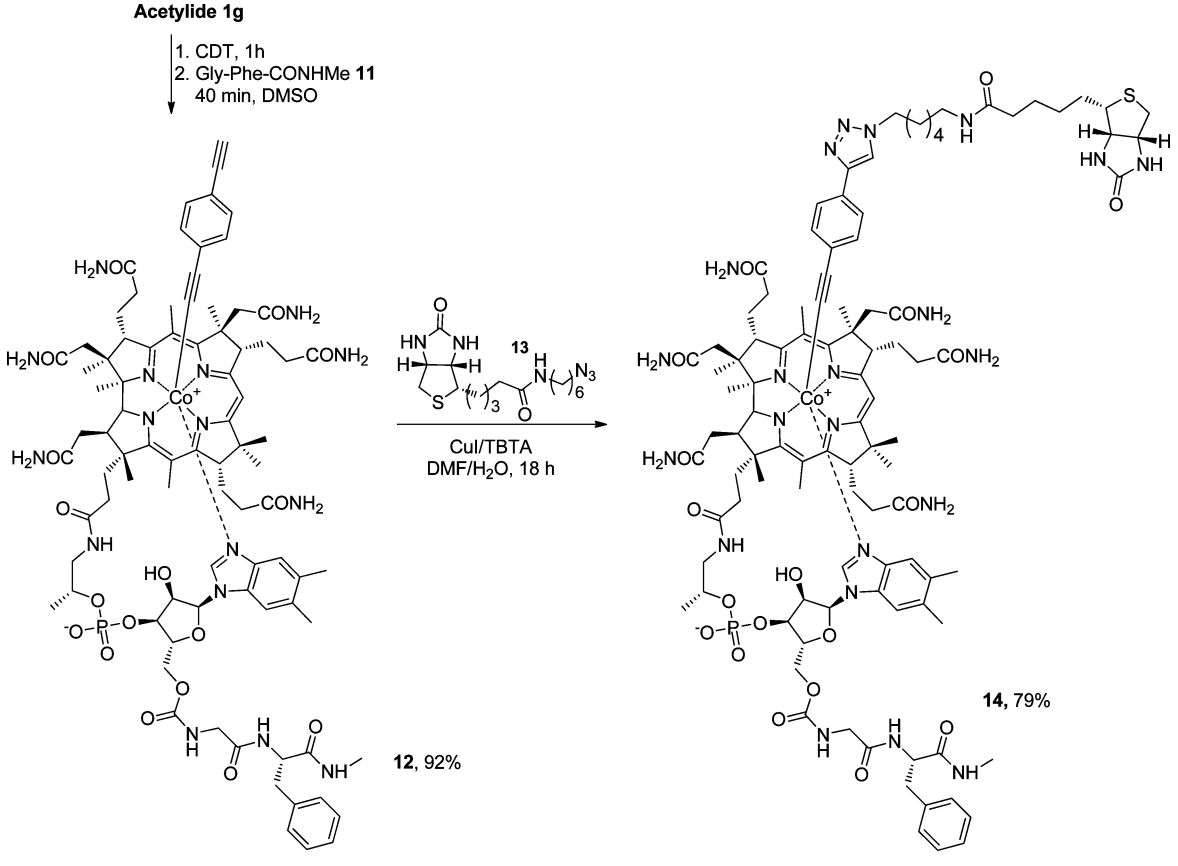


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Table 4. HPLC Method

time (min)	solvent A (%)	solvent B (%)
initial	99	1
15	30	70
30	30	70

1H), 7.10 (s, 1H), 7.70 (s, 1H), 6.69 (s, 1H), 6.90 (s, 1H), 6.88 (s, 1H), 6.86 (s, 1H), 6.77 (s, 1H), 6.67 (s, 1H), 6.53 (s, 1H), 6.50 (s, 1H), 6.36−6.33 (m, 1H), 6.28 (s, 1H), 6.08 (s, 1H), 5.87 (s, 1H), 4.57 (d, 1H, J = 6.7 Hz), 4.54−4.43 (m, 1H), 4.20−4.06 (m, 2H), 3.94−3.85 (m, 2H), 3.78 (dd, 1H, J = 5.4 and 9.8 Hz), 3.66−3.49 (m, 3H), 3.07 (d, 1H, J = 10.9 Hz), 2.78−2.56 (m, 3H), 2.48−2.35 (m, 4H), 2.46 (s, 3H), 2.39 (s, 3H), 2.32−2.21 (m 3H), 2.18 (s, 3H), 2.17 (s, 3H), 2.13−1.99 (m 3H), 1.97−1.85 (m, 1H), 1.83−1.69 (m, 5H), 1.67 (s, 3H), 1.64−1.51 (m, 2H), 1.35 (s, 3H), 1.28−1.20 (m, 2H), 1.18 (s, 6H), 1.05 (d, 3H, J = 6.1 Hz), 0.97 (s, 4H), 0.32 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 178.5, 177.3, 174.3, 174.0, 173.7, 173.3, 173.1, 173.04, 173.00, 171.7, 171.4, 165.1, 164.4, 143.1, 137.2, 132.6, 132.5, 131.7, 131.4, 131.2, 130.2, 119.2, 117.3, 111.6, 107.9, 105.6, 103.2, 100.6, 93.7, 85.7, 84.7, 81.8, 75.2, 74.8, 71.1, 69.7, 62. 3, 58.7, 55.2, 54.0, 53.4, 50.4, 47.7, 46.8, 45.9, 42.1, 39.8, 39.6, 39.5, 38.4, 35.7, 34.2, 32.5, 32.4, 31.8, 31.6, 30.3, 27.7, 26.4, 26.0, 20.8, 20.7, 20.4, 20.3, 20.1, 19.2, 17.0, 16.9, 16.1, 15.4; UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 551 (6.3 × 10^3), 522 (5.2 × 10^3), 363 (1.3 × 10^4), 266 (2.1 × 10^4), 218 (2.7 × 10^4); HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{71}H_{93}N_{14}O_{14}PCo$ 1455.6065, found 1455.6056. Anal. Calcd for $C_{71}H_{92}CoN_{14}O_{14}P{\cdot}8H_2O$: C, 53.31; H, 6.81; N, 12.26. Found: C, 53.37; H, 6.82; N, 12.26. HPLC: t_R = 11.50 min.

Acetylide ***1c:*** yield 105 mg (70%); red powder, mp >225 °C dec; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.70 (s, 1H), 7.59 (s, 1H), 7.55 (s, 1H), 7.51 (s, 1H), 7.45 (s, 1H), 7.43 (s, 1H), 7.33 (s, 1H), 7.31 (s, 1H), 7.15 (s, 1H), 7.11 (s, 1H), 7.06 (s, 1H), 6.96 (s, 1H), 6.94 (s, 1H), 6.92 (s, 1H), 6.91 (s, 1H), 6.77 (s, 1H), 6.53 (s, 1H), 6.51 (s, 1H), 6.39−6.31 (m, 1H), 6.28 (s, 1H), 6.08 (s, 1H), 5.87 (s, 1H), 4.58 (d, 1H, J = 6.3 Hz), 4.53−4.44 (m, 1H), 4.18 (d, 1H, J = 11.2 Hz), 3.97−3.86 (m, 2H), 3.83−3.72 (m, 1H), 3.65−3.50 (m, 3H), 3.07 (d, 1H, J = 10.8 Hz), 2.78−2.54 (m, 3H), 2.48−2.34 (m, 4H), 2.47 (s, 3H), 2.40 (s, 3H), 2.32- 2.22 (m, 5H), 2.19 (s, 3H), 2.17 (s, 3H), 2.15−2.01 (m, 5H), 1.97−1.86 (m, 1H), 1.84−1.71 (m, 4H), 1.67 (s, 3H), 1.63−1.52 (m, 2H), 1.36 (s, 3H), 1.29−1.21 (m, 2H), 1.19 (s, 6H), 1.05 (d, 3H, J = 6.1 Hz), 0.98 (s, 4H), 0.32 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 178.5, 177.2, 174.2, 174.0, 173.7, 173.3, 173.1, 173.0, 172.9, 171.7, 171.4, 165.1, 164.4, 143.2, 137.2, 132.4, 131.2, 131.2, 131.2, 130.2, 126.1, 125.8, 125.7, 125.6, 125.5, 123.5, 117.3, 111.6, 105.7, 103.2, 99.9, 93.6, 85.7, 84.7, 81.8, 75.1, 74.8, 71.1, 69.7, 62.3, 58.7, 55.2, 54.0, 53.4, 50.3, 49.0, 47.7, 46.8, 45.9, 42.2, 38.4, 35.7, 34.2, 32.5, 32.4, 31.8, 31.6, 30.3, 27.7, 26.4, 26.0, 20.7, 20.4, 20.3, 20.2, 19.2, 16.9, 16.8, 16.1, 15.4; UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 551 (6.3 × 10^3), 522 (5.0 × 10^3), 363 (1.2 × 10^4), 274 (2.6 × 10^4), 213 (3.5 × 10^4); HRMS (ESI) m/z [M + 2Na]$^{2+}$ calcd for $C_{71}H_{92}N_{13}O_{14}F_3PCoNa_2$ 771.7846, found 771.7833. Anal. Calcd for $C_{71}H_{92}N_{13}O_{14}$ $F_3PCo{\cdot}6H_2O$: C, 53.08; H, 6.52; N, 11.33. Found: C, 52.99; H, 6.57; N, 11.09. HPLC: t_R = 13.25 min.

Acetylide ***1d:*** yield 121 mg (81%); red powder, mp >190 °C dec; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.66 (s, 1H), 7.62 (s, 1H), 7.54 (s, 2H), 7.49 (s, 1H), 7.44 (d, 1H, J = 7.4 Hz), 7.37−7.26 (m, 3H), 7.21−7.10 (m, 2H), 7.09 (s, 1H), 7.03 (s, 1H), 6.91−6.86 (m, 3H), 6.77 (s, 1H), 6.66 (s, 1H), 6.52 (s, 1H), 6.50 (s, 1H), 6.32 (s, 1H), 6.27 (s, 1H), 6.07 (s, 1H), 5.87 (s, 1H), 4.69 (d, 1H, J = 7.1 Hz), 4.54−4.44 (m, 1H), 4.22−4.07 (m, 2H), 3.95−3.85 (m, 2H), 3.84−3.76 (m, 1H), 3.64−3.51 (m, 3H), 3.08 (d, 1H, J = 10.7 Hz), 2.82−2.56 (m, 3H), 2.49−2.20 (m, 7H), 2.47 (s, 3H), 2.40 (s, 3H), 2.18 (s, 3H), 2.17 (s, 3H), 2.15−2.00 (m, 7H), 1.98−1.84 (m, 1H), 1.83−1.58 (m, 4H), 1.59−1.49 (m 2H), 1.36 (s, 3H), 1.26−1.17 (m, 2H), 1.16 (s, 3H), 1.15 (s, 3H), 1.05 (d, 3H, J = 5.9 Hz), 1.02 (s, 3H), 0.97−0.85 (m, 1H), 0.32 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 178.5, 177.1, 174.3, 174.0, 173.8, 173.36, 173.3, 173.1, 172.9, 171.8, 171.5, 165.2, 164.3, 143.1, 137.2, 134.7, 132.4, 132.1, 131.1, 130.2, 128.7, 128.5, 128.2, 128.0, 125.8, 125.1, 125.0, 123.0, 117.4, 111.6, 105.6, 103.2, 97.2, 93.6, 85.8, 84.8, 81.8, 75.1, 71.1, 69.7, 65.4, 62.3, 54.9, 53.7, 53.4, 50.3, 47.6, 46.8, 45.9, 42.1, 38.4, 35.8, 34.2, 32.5, 32.2, 31.9, 31.7, 30.3, 27.7, 26.5, 26.1, 20.7, 20.4, 20.3, 20.2, 19.1, 17.0, 16.9, 16.1,15.4; UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 551 (4.8 × 10^3), 520 (4.0 × 10^3), 369 (1.6 × 10^4), 266 (2.5 × 10^4), 218 (3.6 × 10^4); HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{71}H_{93}N_{13}O_{14}F_3PCo$ 1498.5987, found 1498.5964. Anal. Calcd for $C_{71}H_{92}N_{13}O_{14}CoF_3P{\cdot}8H_2O$: C, 51.92; H, 6.63; N, 11.09. Found: C, 52.07; H, 6.79; N, 11.10. HPLC: t_R = 12.30 min.

Acetylide ***1e:*** yield 106 mg (71%); red powder, mp >230 °C dec; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.65 (s, 1H), 7.59 (s, 1H), 7.50 (s, 3H), 7.33 (s, 1H), 7.30 (s, 3H), 7.27 (d, 2H, J = 8.4 Hz), 7.16 (s, 1H), 7.09 (s, 1H), 7.05 (s, 1H), 6.96 (s, 1H), 6.77 (s, 1H), 6.68 (d, 2H, J = 8.4 Hz), 6.66 (s, 1H), 6.52 (s, 1H), 6.50 (s, 1H), 6.35 (s, 1H), 6.27 (s, 1H), 5.86 (s, 1H), 4.57 (d, 1H, J = 6.5 Hz), 4.49 (s, 1H), 4.17 (d, 1H, J = 11.0 Hz), 4.11 (s, 1H), 3.90 (bs, 2H), 3.77 (dd, 1H, J = 5.3 and 9.9 Hz), 3.67−3.47 (m, 3H), 3.06 (d, 1H, J = 11.0 Hz), 2.81−2.58 (m, 3H), 2.48−2.34 (m, 2H), 2.46 (s, 3H), 2.39 (s, 3H), 2.31−2.21 (m, 3H), 2.19 (s, 3H), 2.16 (s, 3H), 2.15−1.99 (m, 4H), 1.97−1.84 (m, 1H), 1.83−1.69 (m, 5H), 1.67 (s, 3H), 1.64−1.51 (m, 3H), 1.35 (s, 3H), 1.28−1.20 (m, 2H), 1.17 (s, 6H), 1.06 (d, 3H, J = 5.3 Hz), 0.97 (s, 4H), 0.31 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 178.4, 177.1, 174.2, 174.0, 173.7, 173.35, 173.2, 173.1, 172.9, 171.8, 171.4, 165.0, 164.3, 143.1, 137.3, 132.6, 132.4, 131.5, 131.2, 130.2, 126.3, 118.7, 117.3, 111.5, 110.0, 105.6, 103.1, 99.4, 93.6, 85.7, 84.7, 81.7, 75.2, 74.8, 71.1, 69.7, 62.3, 58.7, 55.1, 54.0, 53.4, 50.4, 47.6, 46.7, 45.9, 42.1, 38.3, 35.7, 34.2, 32.4, 31.8, 31.6, 30.4, 27.7, 26.4, 26.0, 20.7, 20.4, 20.3, 20.2, 19.2, 16.9, 16.8, 16.1, 15.4; UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 551 (4.3 × 10^3), 522 (4.2 × 10^3), 364 (1.0 × 10^4), 267 (2.4 × 10^4), 213 (3.3 × 10^4); HRMS (ESI) m/z [M + 2H]$^{2+}$ calcd for $C_{70}H_{94}N_{13}O_{14}PCoBr$ 754.7631, found 754.7642. Anal. Calcd for $C_{70}H_{92}N_{13}O_{14}PCoBr{\cdot}10H_2O$: C, 49.76; H, 6.68; N, 10.78. Found: C, 49.60; H, 6.30; N, 10.72. HPLC: t_R = 12.95 min.

Acetylide ***1g.*** This compound was prepared on aa 0.30 mmol scale using vitamin B_{12} (408 mg, 0.30 mmol), CuOAc (36 mg, 0.30 mmol), 1,4-diethynylbenzene (378 mg, 3.0 mmol), and DBU (66 μL, 0.45 mmol) in DMA (12.0 mL). The reaction was stopped after 2.5 h to avoid dimer formation: yield 305 mg (70%). red powder, mp >230 °C dec; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.69 (s, 1H), 7.60 (s, 1H), 7.55 (s, 1H), 7.51 (s, 1H), 7.34 (s, 1H), 7.30 (s, 1H), 7.19 (d, 2H, J = 8.2 Hz), 7.15 (s, 1H), 7.10 (s, 1H), 7.05 (s, 1H), 6.96 (s, 1H), 6.90 (s, 1H), 6.77 (s, 1H), 6.74 (d, 2H, J = 8.2 Hz), 6.67 (s, 1H), 6.53 (s, 1H), 6.50 (s, 1H), 6.36−6.29 (m, 1H), 6.28 (s, 1H), 6.06 (s, 1H), 5.86 (s, 1H), 4.59 (d, 1H, J = 6.4 Hz), 4.54−4.43 (m, 1H), 4.18 (d, 1H, J = 11.1 Hz), 4.13 (s, 1H), 4.12−4.06 (m, 1H), 3.97−3.86 (m, 2H), 3.81−3.73 (m, 1H), 3.65−3.51 (m, 3H), 3.06 (d, 1H, J = 11.1 Hz), 2.78−2.56 (m, 3H), 2.49−2.33 (m, 4H), 2.46 (s, 3H), 2.39 (s, 3H), 2.33−2.21 (m, 3H), 2.18 (s, 3H), 2.17 (s, 3H), 2.13−1.97 (m, 3H), 1.95−1.84 (m, 1H), 1.84−1.71 (m, 5H), 1.67 (s, 3H), 1.63−1.51 (m, 3H), 1.35 (s, 3H), 1.28−1.21 (m, 2H), 1.18 (s, 3H), 1.17 (s, 3H), 1.05 (d, 3H, J = 6.1 Hz), 0.97 (s, 4H), 0.32 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 177.9, 176.6, 173.7, 173.6, 173.3, 172.9, 172.7, 172.6, 172.4, 171.4, 171.0, 164.6, 163.9, 142.7, 136.8, 132.0, 131.5, 130.7, 130.4, 129.8, 127.2, 118.4, 116.9, 111.1, 105.2, 102.7, 100.0, 93.2, 85.3, 84.3, 83.3, 81.4, 81.3, 74.6, 74.3, 70.6, 69.3, 61.8, 58.3, 54.7, 53.5, 52.9, 49.9, 48.6, 47.2, 46.3, 45.4, 41.7, 41.6, 20.3, 19.90, 19.88, 19.7, 18.8, 16.5, 16.4, 15.7, 14.9; UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 550 (8.1 × 10^3), 522 (6.2 × 10^3), 364 (1.6 × 10^4), 283 (4.3 × 10^4), 218 (4.4 × 10^4); HRMS (ESI) m/z [M + 2Na]$^{2+}$ calcd for $C_{72}H_{93}N_{13}O_{14}PCoNa_2$ 749.7909, found 749.7908. Anal. Calcd for $C_{72}H_{93}N_{13}O_{14}PCo{\cdot}9H_2O$: C, 53.49; H, 6.92; N, 11.26. Found: C, 53.27; H, 6.77; N, 11.33. HPLC: t_R = 12.27 min.

Acetylide ***1h:*** yield 132 mg (91%); red powder, mp >205 °C dec; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.21 (s, 1H), 7.20 (s, 1H), 7.05 (d, 2H, J = 8.1 Hz), 6.82 (d, 2H, J = 8.1 Hz), 6.63 (s, 1H), 6.25 (d, 1H, J = 2.8 Hz), 5.95 (s, 1H), 4.70 (m, 1H), 4.58 (d, 1H, J = 7.7 Hz), 4.45 (s, 2H), 4.42 (d, 2H, J = 11.5 Hz), 4.34 (m, 1H), 4.20 (m, 1H), 4.10 (m, 1H), 3.96−3.89 (m, 1H), 3.80−3.73 (m, 1H), 3.66 (m, 1H), 3.53 (dd, 1H, J = 4.8, J = 10.4 Hz), 3.20 (d, 1H, J = 9.9 Hz), 2.89−2.78 (m, 2H),

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2.64−2.47 (m, 6H), 2.57 (s, 3H), 2.55 (s, 3H), 2.47−2.37 (m, 3H), 2.36−2.16 (m, 4H), 2.28 (s, 3H), 2.26 (s, 3H), 2.10−1.94 (m, 4H), 1.93−1.87 (m, 2H), 1.86 (s, 3H), 1.82−1.68 (m, 2H), 1.45 (s, 3H), 1.40−1.31 (m, 1H), 1.34 (s, 3H), 1.28 (s, 3H), 1.24 (d, 3H, J = 6.2 Hz), 1.12 (s, 3H), 0.49 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 179.7, 178.1, 177.7, 177.5, 176.9, 176.0, 175.7, 175.0, 174.6, 173.9, 166.4, 165.6, 143.8, 140.5, 138.8, 135.0, 133.2, 131.9, 131.7, 127.8, 126.8, 118.6, 112.0, 108.4, 104.7, 95.3, 87.8, 86.2, 83.4, 75.7, 73.7, 70.8, 64.8, 62.3, 59.9, 57.0, 55.2, 52.3, 44.3, 43.3, 39.9, 35.5, 33.3, 33.0, 32.7, 29.6, 27.5, 27.4, 20.8, 20.3, 20.0, 17.5, 17.1, 16.5, 16.1; UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 551 (5.2 × 10^3), 522 (4.2 × 10^3), 363 (1.0 × 10^4), 263 (2.3 × 10^4), 218 (2.4 × 10^4); HRMS (ESI) m/z [M + 2Na]$^{2+}$ calcd for $C_{71}H_{95}N_{13}O_{15}PCoNa_2$ 752.7962, found 752.7953. Anal. Calcd for $C_{71}H_{95}N_{13}O_{15}CoP{\cdot}11H_2O$: C, 51.41; H, 7.11; N, 10.98. Found: C, 51.58; H, 6.92; N, 10.74. HPLC: t_R = 10.50 min.

Acetylide ***2a:*** yield 72 mg (52%); red powder, mp >190 °C dec; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.58 (s, 1H), 7.55 (s, 1H), 7.53 (s, 1H), 7.50 (s, 1H), 7.44 (s, 1H), 7.32 (s, 1H), 7.25 (s, 1H), 7.10 (s, 1H), 7.04 (s, 2H), 6.94 (s, 1H), 6.89 (s, 1H), 6.75 (s, 1H), 6.62 (s, 1H), 6.60 (s, 1H), 6.46 (s, 1H), 6.25 (s, 1H), 6.23 (s, 1H), 6.01 (s, 1H), 5.76 (s, 1H), 4.54−4.41 (m, 2H), 4.28 (d, 1H, J = 7.4 Hz), 4.20 (d, 1H, J = 11.1 Hz), 4.14−4.04 (m, 2H), 3.91 (s, 1H), 3.88−3.82 (m, 2H), 3.71 (s, 2H), 3.61−3.50 (m, 5H), 3,02 (d, 1H, J = 10.8 Hz), 2.76−2.54 (m, 3H), 2.45−2.21 (m, 3H), 2.42 (s, 3H), 2.37 (s, 3H), 2.15 (s, 3H), 2.13 (s, 3H), 2.13−1.97 (m, 4H), 1.95−1.84 (m, 2H), 1.81−1.69 (m, 3H), 1.67 (s, 3H), 1.65−1.46 (m, 5H), 1.30 (s, 3H), 1.25−1.20 (m, 2H), 1.18 (s, 6H), 1.05 (d, 3H, J = 5.9 Hz), 0.98 (s, 4H), 0.25 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 177.8, 176.6, 174.0, 173.9, 173.8, 173.6, 173.4, 173.1, 172.5, 172.1, 171.6, 164.7, 164.1, 143.1, 137.4, 132.3, 131.0, 130.2, 117.5, 111.4, 98.7, 93.3, 85.7, 84.7, 81.7, 75.0, 74.5, 71.2, 69.6, 62.2, 55.6, 54.2, 53.3, 50.7, 50.2, 47.4, 46.6, 45.8, 42.4, 42.0, 38.2, 35.7, 34.3, 32.4, 31.9, 30.4, 27.6, 26.4, 26.1, 20.7, 20.5, 20.3, 20.1, 19.5, 17.0, 16.9, 16.2, 15.5; UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 547 (5.4 × 10^3), 522 (4.2 × 10^3), 367 (9.9 × 10^3), 269 (2.0 × 10^4), 215 (3.6 × 10^4); HRMS (ESI) m/z [M + Na]$^+$ calcd for $C_{65}H_{91}N_{13}O_{15}PCoNa$, 1406.5725, found 1406.5713. Anal. Calcd for $C_{65}H_{91}N_{13}O_{15}CoP{\cdot}11H_2O$: C, 49.33; H, 7.20; N, 11.51. Found: C, 49.27; H, 6.85; N, 11.59. HPLC: t_R = 9.08 min.

Acetylide ***2b:*** yield 75 mg (53%); red powder, mp >220 °C dec; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.59 (s, 1H), 7.55 (s, 1H), 7.52 (s, 1H), 7.49 (s, 2H), 7.31 (s, 1H), 7.26 (s, 1H), 7.09 (s, 1H), 7.06 (s, 1H), 7.01 (s, 1H), 6.92 (s, 1H), 6.88 (s, 1H), 6.74 (s, 1H), 6.62 (s, 1H), 6.50 (s, 1H), 6.47 (s, 1H), 6.31 (s, 1H), 6.24 (s, 1H), 6.01 (s, 1H), 5.77 (s, 1H), 4.52−4.43 (m, 1H), 4.30 (d, 1H, J = 6.5 Hz), 4.26−4.17 (m, 2H), 4.15−4.04 (m, 1H), 3.95−3.85 (m, 2H), 3.70−3.62 (m, 1H), 3.61−3.45 (m, 3H), 3.03 (d, 1H, J = 10.8 Hz), 2.78−2.56 (m, 3H), 2.47−2.33 (m, 5H), 2.44 (s, 3H), 2.38 (s, 3H), 2.31−2.22 (m, 3H), 2.17 (s, 3H), 2.15 (s, 3H), 2.12−1.98 (m, 2H), 1.91−1.70 (m, 5H), 1.68 (s, 3H), 1.64−1.47 (m, 3H), 1.33 (s, 3H), 1.27−1.23 (m, 2H), 1.21 (s, 3H), 1.20 (s, 3H), 1.06 (d, 3H, J = 5.3), 1.01 (s, 3H), 0.99 (s, 7H), 0.27 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 177.3, 176.0, 173.5, 173.3, 173.2, 173.0, 172.9, 172.6, 171.9, 171.6, 171.1, 164.1, 163.6, 142.6, 136.9, 131.7, 130.4, 129.8, 117.1, 110.9, 105.1, 102.5, 92.8, 85.2, 84.2, 81.3, 74.6, 74.0, 70.6, 69.2, 63.7, 61.8, 57.9, 55.0, 53.6, 52.9, 49.6, 46.9, 46.1, 45.4, 41.6, 37.7, 35.2, 33.7, 33.2, 33.1, 32.0, 31.5, 31.0, 29.9, 27.2, 25.9, 25.6, 20.3, 19.8, 19.6, 19.0, 16.5, 16.3, 15.6, 14.9; UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 545 (5.1 × 10^3), 522 (4.1 × 10^3), 368 (9.2 × 10^3), 269 (2.0 × 10^4), 218 (3.6 × 10^4); HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{67}H_{96}N_{13}O_{15}PCo$ 1412.6218, found 1412.6202. Anal. Calcd for $C_{67}H_{95}N_{13}O_{15}PCo{\cdot}7H_2O$: C, 52.30; H, 7.14; N, 11.83. Found: C, 52.28; H, 6.98; N, 11.58. HPLC: t_R = 9.98 min.

Acetylide ***2d.*** This compound was prepared on a 0.05 mmol scale: yield 68 mg (91%); red powder, mp >190 °C dec; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.20 (s, 1H), 7.18 (s, 2H), 7.14 (s, 1H), 6.71 (d, 2H, J = 8.6 Hz), 6.55 (s, 1H), 6.22 (d, 1H, J = 3.1 Hz), 5.92 (s, 1H), 4.64 (td, 1H, J = 4.0 and 8.4 Hz), 4.54 (d, 2H, J = 3.8 Hz), 4.52−4.46 (m, 1H), 4.39 (s, 2H), 4.35−4.26 (m, 1H), 4.19−4.14 (m, 2H), 4.07 (m, 1H), 3.90 (dd, 1H, J = 3.2 and 12.5 Hz), 3.74 (dd, 1H, J = 4.3 and 12.6 Hz), 3.67−3.60 (m, 1H), 3.51−3.45 (m, 1H), 3.19 (d, 1H, J = 10.3 Hz), 2.84 (dd, 1H, J = 9.1 and 14.0 Hz), 2.74 (dt, 1H, J = 5.7 and 11.4 Hz), 2.59−2.44 (m, 6H), 2.51 (s, 3H), 2.50 (s, 3H), 2.44−2.27 (m, 4H), 2.27−2.22 (m, 2H), 2.25 (s, 6H), 2.21−2.08 (m, 2H), 2.08−1.99 (m, 1H), 1.98−1.84 (m, 5H), 1.82 (s, 3H), 1.79−1.63 (m, 2H), 1.42 (s, 3H), 1.36−1.28 (m, 1H), 1.29 (s, 3H), 1.23 (d, 3H, J = 6.3 Hz), 1.13 (s, 3H), 1.08 (s, 3H), 0.41 (s, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 179.6, 178.0, 177.8, 177.5, 176.9, 176.2, 176.0, 175.7, 175.1, 174.5, 173.8, 166.2, 165.6, 158.6, 143.7, 138.8, 134.9, 134.7, 133.1, 131.6, 129.8, 118.6, 115.8, 112.0, 108.2, 104.7, 96.6, 95.2, 87.8, 86.2, 75.8, 75.6, 73.6, 70.7, 65.0, 62.7, 59.8, 57.7, 57.0, 56.8, 55.0, 52.0, 48.1, 46.7, 39.9, 36.4, 35.5, 33.2, 32.9, 32.0, 29.6, 27.5, 20.8, 20.5, 20.2, 20.1, 20.0, 17.5, 17.2, 16.6, 16.2; UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 551 (5.3 × 10^3), 520 (4.2 × 10^3), 363 (1.1 × 10^4), 263 (2.1 × 10^4), 216 (3.4 × 10^4); HRMS (ESI) m/z [M + 2Na]$^{2+}$ calcd for $C_{72}H_{97}N_{13}O_{16}PCoNa_2$ 767.8015, found 767.7996. Anal. Calcd for $C_{72}H_{97}N_{13}O_{16}PCo{\cdot}11H_2O$: C, 51.21; H, 7.10; N, 10.78. Found: C, 51.30; H, 6.97; N, 10.83. HPLC: t_R = 11.72 min.

General Procedure for Synthesis of Compounds 3a,b. CuI (1 mg, 0.005 mmol) and TBTA (5 mg, 0.01 mmol) were suspended in a DMF/H_2O mixture (0.7 mL, 3/1 v/v) and stirred until a clear, yellowish solution was formed. Acetylide **3** (30 mg, 0.02 mmol) and the respective azide (0.024 mmol) were added, and the reaction mixture was stirred for 16 h at room temperature. To the reaction mixture were added MeOH (1 mL) and Et_2O (15 mL), and the precipitate was centrifuged and dried in air. The crude product was dissolved in ice-cold water (5 mL) and centrifuged to remove the copper complexes. The solution, containing the crude product, was then charged on an RP C18 column and eluted with H_2O/MeCN (gradually from 10% to 40% of MeCN). The most intense band was collected, solvents were evaporated, and the residue was dissolved in MeOH (1 mL), precipitated with Et_2O (15 mL), centrifuged, and dried overnight under reduced pressure at 50 °C to give the respective product.

Compound ***3a:*** yield 24 mg (75%); red powder, mp >230 °C dec; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.48 (s, 1H), 7.61 (s, 1H), 7.55 (s, 1H), 7.53 (s, 1H), 7.50 (s, 1H), 7.45 (s, 1H), 7.39−7.27 (m, 7H), 7.25 (m, 1H), 7.15 (s, 1H), 7.10 (s, 1H), 7.04 (s, 1H), 6.94 (s, 1H), 6.89 (s, 1H), 6.80 (s, 1H), 6.79 (s, 1H), 6.76 (s, 1H), 6.64 (s, 1H), 6.51 (s, 1H), 6.50 (s, 1H), 6.24 (s, 1H), 6.06 (bs, 1H), 5.86 (s, 1H), 5.58 (s, 2H), 4.58 (bs, 2H), 4.21 (d, 1H, J = 11.0 Hz), 4.13 (bs, 1H), 3.96 (bs, 1H), 3.90 (bs, 1H), 3.80−3.66 (m, 1H), 3.64−3.45 (m, 2H), 3.04 (d, 1H, J = 10.6 Hz), 2.81−2.54 (m, 3H), 2.47−2.30 (m, 8H), 2.46 (s, 3H), 2.38 (s, 3H), 2.29−2.19 (m, 8H), 2.15 (s, 3H), 2.13−1.98 (m, 2H), 1.95−1.85 (m, 1H), 1.84−1.69 (m, 3H), 1.66 (s, 3H), 1.61−1.59 (m, 2H), 1.34 (s, 3H), 1.28−1.20 (m, 2H), 1.18 (s, 3H), 1.16 (s, 3H), 1.11−1.02 (m, 3H), 0.97 (s, 4H), 0.32 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 177.9, 176.5, 173.7, 173.6, 173.3, 172.9, 172.7, 172.6, 172.4, 171.4, 171.0, 164.6, 163.9, 146.3, 142.6, 136.8, 135.9, 132.0, 130.8, 129.8, 128.8, 128.2, 127.9, 127.7, 126.2, 124.7, 121.3, 116.9, 110.9, 109.5, 93.2, 85.3, 84.2, 81.3, 74.3, 69.2, 58.2, 54.6, 53.6, 52.9, 49.9, 47.2, 46.2, 41.8, 41.6, 37.8, 35.2, 33.8, 32.0, 31.2, 29.9, 27.2, 26.0, 25.6, 20.2, 19.9, 19.7, 18.8, 16.5, 16.4, 15.7, 14.9; UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 551 (5.7 × 10^3), 520 (4.3 × 10^3), 364 (1.5 × 10^4), 288 (3.4 × 10^4), 215 (3.0 × 10^4); HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{79}H_{101}N_{16}O_{14}PCo$ 1587.6753, found 1587.6737. Anal. Calcd for $C_{79}H_{100}N_{16}O_{14}PCo{\cdot}6H_2O$: C, 55.95; H, 6.66; N, 13.22. Found: C, 56.15; H, 6.70; N, 13.17. HPLC: t_R = 13.05 min.

Compound ***3b:*** yield 25 mg (78%); red powder, mp >200 °C dec; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.42 (s, 1H), 7.65 (s, 1H), 7.58 (s, 1H), 7.54 (d, 2H, J = 8.2 Hz), 7.50 (s, 2H), 7.32 (s, 1H), 7.29 (s, 1H), 7.15 (s, 1H), 7.10 (s, 1H), 7.04 (s, 1H), 6.96 (s, 1H), 6.89 (s, 1H), 6.81 (d, 2H, J = 8.2 Hz), 6.76 (s, 1H), 6.65 (s, 1H), 6.50 (bs, 2H), 6.33 (s, 1H), 6.26 (s, 1H), 6.04 (s, 1H), 5.86 (s, 1H), 4.65 (t, 1H, J = 5.2 Hz), 4.59 (d, 1H, J = 7.2 Hz), 4.51 (t, 2H, J = 5.0 Hz), 4.49−4.44 (m, 1H), 4.22 (d, 1H, J = 11.2 Hz), 4.15−4.05 (m, 1H), 3.93−3.84 (m, 2H), 3.81 (t, 2H, J = 5.1 Hz), 3.80−3.74 (m, 1H), 3.64−3.50 (m, 3H), 3.48−3.39 (m, 4H), 3.06 (d, 1H, J = 11.1 Hz), 2.76−2.55 (m, 3H), 2.48−2.32 (m, 5H), 2.47 (s, 3H), 2.39 (s, 3H), 2.31−2.19 (m, 4H), 2.17 (s, 3H), 2.16 (s, 3H), 2.12−1.99 (m, 2H), 1.96−1.85 (m, 2H), 1.85−1.70 (m, 4H), 1.67 (s, 3H), 1.63−1.51 (m, 2H), 1.34 (s, 3H),

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1.28−1.21 (m, 2H), 1.19 (s, 3H), 1.17 (s, 3H), 1.05 (d, 3H, J = 6.1 Hz), 0.98 (s, 4H), 0.32 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 178.3, 177.0, 174.1, 174.0, 173.8, 173.4, 173.2, 173.1, 172.8, 171.9, 171.5, 165.0, 164.3, 146.3, 143.1, 137.3, 132.4, 131.2, 131.1, 130.2, 128.3, 126.6, 125.3, 122.0, 117.4, 111.5, 110.0, 105.7, 103.2, 100.4, 93.6, 85.7, 84.7, 75.1, 74.8, 72.5, 71.1, 69.0, 65.4, 62.3, 60.5, 55.1, 54.0, 53.4, 50.4, 50.0, 47.6, 46.8, 45.9, 42.2, 38.3, 35.7, 34.2, 32.5, 32.4, 31.8, 31.6, 30.3, 27.7, 26.5, 26.0, 20.8, 20.4, 20.3, 20.2, 20.1, 19.2, 17.0, 16.9, 16.2, 15.4; UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 550 (5.5 × 10^3), 522 (4.1 × 10^3), 364 (1.4 × 10^4), 262 (2.1 × 10^4), 218 (2.7 × 10^4); HRMS (ESI) m/z [M + 2H]$^{2+}$ calcd for $C_{76}H_{104}N_{16}O_{16}PCo$ 793.3437, found 793.3428. Anal. Calcd for $C_{76}H_{102}N_{16}O_{16}PCo\cdot 12H_2O$: C, 50.66; H, 7.05; N, 12.44. Found: C, 50.71; H, 6.70; N, 12.17. HPLC: t_R = 10.62 min.

Synthesis of Compound 5. Compound **5** was obtained from mesylate **4**[22] (215 mg, 0.15 mmol) according to the procedure described for compound **1g**: yield 177 mg (77%); red powder, mp >230 °C dec; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.23 (s, 2H), 7.16 (d, 2H, J = 8.3 Hz), 6.79 (d, 2H, J = 8.3 Hz), 6.62 (s, 1H), 6.28 (d, 1H, J = 2.8 Hz), 5.97 (s, 1H), 4.63−4.66 (m, 1H), 4.50 (dd, 1H, J = 3.4 and 11.4 Hz), 4.41−4.26 (m, 3H), 4.19 (m, 1H), 3.66 (d, 1H, J = 13.9 Hz), 3.57 (dd, 1H, J = 5.0 and 10.8 Hz), 3.43 (s, 1H), 3.19 (s, 3H), 3.18 (d, 1H, J = 10.3 Hz), 2.89 (dd, 1H, J = 8.9 and 14.0 Hz), 2.86−2.79 (m, 1H), 2.61−2.71 (m, 6H), 2.57 (s, 3H), 2.55 (s, 3H), 2.51−2.37 (m, 4H), 2.36−2.30 (m, 1H), 2.28 (s, 3H), 2,27 (s, 3H), 2.24−2.16 (m, 1H), 2.16−1.95 (m, 5H), 1.95−1.79 (m, 4H), 1.85 (s, 3H), 1.78−1.65 (m, 1H), 1.45 (s, 3H), 1.37−1.27 (m, 2H), 1.34 (s, 3H), 1.29 (s, 3H), 1.24 (d, 3H, J = 6.3 Hz), 1.11 (s, 3H), 0.49 (s, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 179.8, 178.2, 177.5, 176.9, 176.1, 176.0, 175.6, 174.5, 174.4, 173.9, 166.4, 165.8, 143.7, 138.7, 135.1, 133.4, 132.7, 131.7, 131.6, 128.2, 121.0, 118.6, 112.1, 108.3, 104.9, 102.7, 95.4, 88.1, 86.2, 83.7, 80.8, 75.7, 74.5, 73.6, 70.6, 70.4, 59.8, 57.0, 56.8, 55.4, 52.3, 48.2, 46.6, 44.1, 43.3, 39.9, 37.4, 36.4, 35.3, 33.3, 33.1, 32.64, 32.58, 32.0, 29.3, 27.4, 27.3, 20.82, 20.75, 20.32, 20.27, 20.0, 17.5, 17.2, 16.4, 16.2; UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 550 (8.2 × 10^3), 522 (6.1 × 10^3), 364 (1.7 × 10^4), 283 (4.2 × 10^4), 218 (4.3 × 10^4); HRMS (ESI) m/z [M + 2Na]$^{2+}$ calcd for $C_{73}H_{95}N_{13}O_{16}PCoSNa_2$ 788.7795, found 788.7784. Anal. Calcd for $C_{73}H_{95}N_{13}O_{16}PSCo\cdot 10H_2O$: C, 51.19; H, 6.77; N, 10.63. Found: C, 51.07; H, 6.83; N, 10.55. HPLC: t_R = 12.77 min.

Synthesis of Compound 6. Mesylate **5** (153 mg, 0.10 mmol) was dissolved in HMPA (2 mL), and NaN_3 (50 mg, 0.75 mmol) was added. The reaction mixture was stirred for 16 h at 40 °C. It was then poured into H_2O (50 mL) and washed with DCM (3 × 50 mL). The aqueous phase was extracted with a solution of phenol (20 g) in DCM (20 mL). The organic layer was washed with H_2O (3 × 20 mL) and diluted with DCM to the volume of 200 mL and the product back-extracted with H_2O (3 × 20 mL). After evaporation of water, the residue was dissolved in MeOH (2 mL), precipitated with Et_2O (30 mL), centrifuged, and dried under reduced pressure, affording 140 mg (95%) of azide **6**: red powder, mp >230 °C dec; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.25 (s, 1H), 7.21 (s, 1H), 7.16 (d, 2H, J = 8.2 Hz), 6.80 (d, 2H, J = 8.2 Hz), 6.62 (s, 1H), 6.29 (d, 1H, J = 2.7 Hz), 5.98 (s, 1H), 4.60 (d, 1H, J = 7.6 Hz), 4.38 (d, 1H, J = 11.4 Hz), 4.31 (m, 1H), 4.25−4.15 (m, 2H), 4.03−3.92 (m, 1H), 3.70−3.53 (m, 3H), 3.43 (s, 1H), 3.19 (d, 1H, J = 10.3 Hz), 2.92−2.77 (m, 2H), 2.66−2.50 (m, 5H), 2.57 (s, 3H), 2.56 (s, 3H), 2.49−2.36 (m, 5H), 2.36−2.29 (m, 1H), 2.28 (s, 3H), 2.27 (s, 3H), 2.24−2.14 (m, 1H), 2.14−1.96 (m, 3H), 2.00 (s, 3H), 1.95−1.88 (m, 2H), 1.86 (s, 3H), 1.83−1.64 (m, 2H), 1.46 (s, 3H), 1.40−1.26 (m, 1H), 1.34 (s, 3H), 1.29 (s, 3H), 1.23 (d, 3H, J = 6.3 Hz), 1.12 (s, 3H), 0.49 (s, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 179.9, 178.2, 177.5, 177.3, 176.9, 176.1, 176.0, 175.6, 175.0, 174.4, 174.0, 166.4, 165.8, 143.6, 138.8, 135.1, 133.3, 132.7, 131.9, 131.6, 128.2, 121.0, 118.6, 112.1, 108.3, 104.9, 102.7, 95.4, 88.0, 86.2, 84.2, 82.03, 81.97, 79.5, 75.7, 75.3, 73.51, 73.46, 70.8, 61.5, 59.9, 57.0, 56.8, 55.5, 52.6, 52.3, 48.2, 46.6, 44.1, 43.3, 39.9, 36.4, 35.6, 33.3, 33.1, 32.74, 32.67, 32.1, 29.3, 27.43, 27.38, 20.8, 20.7, 20.31, 20.27, 20.2, 20.1, 20.0, 17.5, 17.1, 16.5, 16.2; UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 551 (8.2 × 10^3), 520 (6.1 × 10^3), 363 (1.5 × 10^4), 283 (4.2 × 10^4), 218 (4.3 × 10^4); HRMS (ESI) m/z [M + Na]$^+$ calcd for $C_{72}H_{92}N_{16}O_{13}PCoNa$ 1501.5997, found 1501.6006. Anal. Calcd for $C_{72}H_{92}N_{16}O_{13}PCo\cdot 10H_2O$: C, 52.11; H, 6.80; N, 13.50. Found: C, 51.94; H, 6.92; N, 13.41. HPLC: t_R = 12.75 min.

One-Pot Synthesis of Compound 8. Compound **6** (30 mg, 0.02 mmol) was dissolved in a DMF/H_2O mixture (0.5 mL, 3/1 v/v) followed by the addition of *endo*-9-hydroxymethylbicyclo[6.1.0]-nonyne (**7**;[38] 5 mg, 0.3 mmol). The reaction mixture was stirred for 16 h at room temperature (full conversion of the starting material was indicated by HPLC analysis). In a separate vial CuI (1 mg, 0.005 mmol) and TBTA (5 mg, 0.01 mmol) were suspended in a DMF/H_2O mixture (0.7 mL, 3/1 v/v) and stirred until a clear, yellowish solution was formed. This solution was transferred to the reaction mixture followed by the addition of benzyl azide (5 mg, 0.04 mmol). After it was stirred for an additional 24 h, the reaction mixture was diluted with MeOH (1 mL) and precipitated with Et_2O (15 mL). The workup and product isolation was performed using the procedure described for compound **3**. Compound **8** was isolated as a mixture of regioisomers: yield 30 mg (84%). red powder, mp > °C dec; ^{1}H NMR (500 MHz, CD_3OD) δ 8.17 (s, 1H), 7.51 (d, 2H, J = 8.0 Hz), 7.39−7.27 (m, 5H), 7.24−7.17 (m, 1.5H), 7.14 (s, 0.5H), 6.91−6.85 (m, 2H), 6.64−6.58 (m, 2H), 6.14 (s, 0.5H), 6.00 (s, 0.5H), 5.99 (s, 0.5H), 5.92 (s, 0.5H), 5.58 (s, 2H), 4.92 (m, 1H), 4.76−4.48 (m, 5H), 4.47−4.29 (m, 2H), 4.25−4.09 (m, 1H), 3.68−3.59 (m, 3H), 3.55 (m, 1H), 3.21−2.95 (m, 4H), 2.95−2.75 (m, 3H), 2.66−2.47 (m, 8H), 2.57 (s, 3H), 2.47−2.30 (m, 5H), 2.27 (s, 3H), 2.26 (s, 3H), 2.25 (s, 3H), 2.23−2.09 (m, 3H), 2.09−1.94 (m, 4H), 1.94−1.86 (m, 2H), 1.85 (s, 3H), 1.79−1.69 (m, 1H), 1.69−1.55 (m, 2H), 1.48−1.37 (m, 2H), 1,43 (s, 1.5H), 1.41 (s, 1,5H), 1.34 (s, 3H), 1.31 (s, 1.5H), 1.30 (s, 1.5H), 1.24 (d, 3H, J = 5.6 Hz),1.14−1.07 (m, 1H), 1.11 (s, 1.5H), 1.10 (s, 1.5H), 1.06−0.91 (m, 1H), 0.92−0.81 (m, 1H), 0.48 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 179.9, 178.1, 177.5, 176.9, 176.2, 176.0, 175.6, 175.0, 174.0, 166.1, 165.8, 148.8, 143.8, 138.7, 136.8, 135.2, 133.5, 132.4, 131.6, 130.0, 129.6, 129.09, 129.05, 127.8, 126.3, 122.1, 118.7, 108.4, 105.1, 102.6, 95.4, 87.6, 86.2, 75.7, 70.6, 59.8, 59.7, 59.6, 57.0, 55.0, 52.35, 52.32, 48.2, 44.2, 43.3, 40.0, 36.4, 33.2, 32.7, 32.0, 31.9, 28.9, 27.4, 26.7, 26.5, 24.4, 24.2, 23.3, 23.1, 22.7, 21.9, 21.7, 20.8, 20.6, 20.5, 20.3, 20.2, 20.1, 20.0, 19.9, 19.8, 17.5, 16.9, 16.6, 16.5; UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 550 (5.9 × 10^3), 522 (4.5 × 10^3), 360 (1.4 × 10^4), 262 (4.2 × 10^4), 218 (4.3 × 10^4); HRMS (ESI) m/z [M+2Na]$^{2+}$ calcd for $C_{89}H_{113}N_{19}O_{14}PCoNa_2$ 903.8784, found 903.8782. Anal. Calcd for $C_{89}H_{113}N_{19}O_{14}PCo\cdot 11H_2O$: C, 54.51; H, 6.94; N, 13.57. Found: C, 54.82; H, 7.33; N, 13.27. HPLC: t_R = 13.57 min.

Synthesis of Compound 9. Compound **9** was obtained from **6** (30 mg, 0.02 mmol) according to the procedure described for compound **3**: yield 27 mg (81%); red powder, mp >230 °C dec; ^{1}H NMR (CD_3OD, 500 MHz) δ 8.17 (s, 1H), 7.51 (d, 2H, J = 8.4 Hz), 7.39−7.29 (m, 5H), 5.25 (s, 1H), 7.22 (s, 1H), 6.89 (d, 2H, J = 8.4 Hz), 6.63 (s, 1H), 6.29 (d, 1H, J = 2.9 Hz), 5.89 (s, 1H), 5.58 (s, 1H), 4.59 (d, 1H, J = 6.8 Hz), 4.41 (d, 1H, J = 11.6 Hz), 4.63−4.25 (m, 1H), 4.24−4.16 (m, 2H), 3.98 (m, 1H), 3.67−3.57 (m, 2H), 3.55 (dd, 1H, J = 4.9 and 10.8 Hz), 3.19 (d, 1H, J = 10.4 Hz), 2.93−2.77 (m, 2H), 2.62−2.53 (m, 6H), 2.58 (s, 3H), 2.56 (s, 3H), 2.53−2.47 (m, 2H), 2.46−2.38 (m, 3H), 2.34 (m, 1H), 2.28 (s, 3H), 2.27 (s, 3H), 2.25−2.15 (m, 1H), 2.13−2.02 (m, 3H), 2.02−1.96 (m, 2H), 1.95−1.89 (m, 2H), 1.86 (s, 3H), 1.84−1.79 (m, 1H), 1.77−1.68 (m, 1H), 1.46 (s, 3H), 1.40−1.31 (m, 2H), 1.34 (s, 3H), 1.30 (s, 3H), 1.23 (d, 3H, J = 6.3 Hz), 1.13 (s, 3H), 0.49 (s, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 179.8, 178.2, 177.5, 177.3, 176.9, 176.1, 176.0, 175.6, 171.0, 174.4, 173.9, 166.4, 165.7, 148.8, 143.7, 138.8, 136.8, 135.1, 133.3, 132.4, 131.6, 130.0, 129.6, 129.1, 129.0, 127.8, 126.3, 122.1, 118.6, 112.1, 108.5, 104.9, 95.4, 88.0, 86.3, 82.0, 75.8, 75.3, 70.8, 59.9, 57.0, 56.0, 55.5, 55.0, 52.6, 52.4, 44.3, 43.3, 39.9, 36.4, 35.6, 33.1, 32.7, 32.1, 29.3, 27.4, 20.8, 20.7, 20.3, 20.2, 20.0, 17.4, 17.1, 16.5, 16.2; UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 552 (5.8 × 10^3), 520 (4.3 × 10^3), 364 (1.5 × 10^4), 283 (4.5 × 10^4), 218 (4.7 × 10^4); HRMS (ESI) m/z [M + Na]$^+$ calcd for $C_{79}H_{99}N_{19}O_{13}PCoNa$ 1634.6637, found 1634.6616. Anal. Calcd for $C_{79}H_{99}N_{19}O_{13}PCo\cdot 7H_2O$: C, 54.57; H, 6.55; N, 15.31. Found: C, 54.90; H, 6.77; N, 14.96. HPLC: t_R = 13.35 min.

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One-Pot Synthesis of Compound 10. CuI (1 mg, 0.005 mmol) and TBTA (5 mg, 0.01 mmol) were suspended in a DMF/H_2O mixture (0.7 mL, 3/1 v/v) and stirred until a clear, yellowish solution was formed. To the solution were added compound **6** (30 mg, 0.02 mmol) and benzyl azide (4 mg, 0.03 mmol), and the reaction mixture was stirred for 18 h at room temperature. After that time full conversion was observed (HPLC analysis); phenylacetylene (4 mg, 0.04 mmol) was added to the reaction mixture, and this mixture was allowed to react for 24 h. The reaction workup and product purification were performed according to the procedure described for compound **3**: yield 22 mg (64%); red powder, mp >230 °C dec; ^{1}H NMR (CD_3OD, 500 MHz) δ 8.74 (s, 1H), 8.18 (s, 1H), 7.89 (s, 1H), 7.87 (s, 1H), 7.51 (d, 2H, J = 8.4 Hz), 7.45−7.39 (m, 2H), 7.37−7.28 (m, 6H), 7.23 (s, 1H), 7.11 (s, 1H), 6.89 (d, 2H, J = 8.4 Hz), 6.61 (s, 1H), 5.99 (s, 1H), 5.97 (d, 1H, J = 2.7 Hz), 5.58 (s, 2H), 5.13 (d, 1H, J = 11.4 Hz), 4.94 (d, 1H, J = 13.1 Hz), 4.64−4.53 (m, 2H), 4.51−4.44 (m, 1H), 4.43−4.32 (m, 2H), 4.09 (m, 1H), 3.70−3.61 (m, 1H), 3.55 (dd, 1H, J = 5.0 and 10.8 Hz), 3.18 (d, 1H, J = 10.2 Hz), 2.91−2.77 (m, 2H), 2.68−2.48 (m, 7H), 2.58 (s, 3H), 2.57 (s, 3H), 2.46−2.37 (m, 3H), 2.36−2.29 (m, 1H), 2.28−2.32 (m, 1H), 2.26 (s, 3H), 2.21 (s, 3H), 2.19−2.10 (m, 1H), 2.06 (d, 1H, J = 13.3 Hz), 2.03−1.88 (m, 4H), 1.85 (s, 3H), 1.84−1.78 (m, 1H), 1.77−1.67 (m, 1H), 1.47 (s, 3H), 1.38−1.28 (m, 2H), 1.34 (s, 3H), 1.30 (s, 3H), 1.25 (d, 3H, J = 6.3 Hz), 1.13 (s, 3H), 0.47 (s, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 179.8, 178.2, 177.5, 176.8, 176.0, 175.6, 175.0, 174.4, 173.9, 166.4, 165.7, 148.8, 143.7, 138.7, 136.8, 135.1, 133.3, 132.4, 131.8, 131.5, 130.0, 129.9, 129.6, 129.3, 129.1, 127.8, 126.9, 126.3, 124.7, 122.1, 118.6, 112.0, 108.4, 104.9, 95.4, 87.7, 86.2, 75.7, 75.2, 73.7, 70.5, 59.8, 57.0, 55.5, 55.0, 52.4, 46.8, 44.3, 43.3, 39.9, 36.4, 35.5, 33.0, 32.7, 32.0, 29.6, 27.3, 20.8, 20.3, 20.2, 20.0, 17.5, 17.2, 16.5, 16.2; UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 552 (5.8×10^3), 520 (4.3×10^3), 364 (1.6×10^4), 283 (4.5×10^4), 218 (4.6×10^4); HRMS (ESI) m/z [M + 2Na]$^{2+}$ calcd for $C_{87}H_{105}N_{19}O_{13}PCoNa_2$ 879.8496, found 879.8469. Anal. Calcd for $C_{87}H_{105}N_{19}O_{13}PCo{\cdot}11H_2O$: C, 54.62; H, 6.69; N, 13.91. Found: C, 54.52; H, 6.81; N, 13.51. HPLC: t_R = 14.18 min.

Synthesis of Compound 12. Compound **1g** (35 mg, 0.02 mmol) was dissolved in anhydrous DMSO (0.6 mL), the solution was heated to 40 °C, and then CDT (20 mg, 0.12 mmol) was added. After 1 h (full conversion, HPLC) the oil bath was removed and to the cooled reaction mixture was added Gly-PheCONHMe (**11**;[40] 10 mg, 0.043 mmol). After 40 min the reaction went to completion (HPLC analysis) and the mixture was diluted with Et_2O (15 mL) and centrifuged. The precipitate was washed with a AcOEt/$CHCl_3$ mixture (15 mL, 1/1 v/v) and Et_2O (2 × 15 mL) and dried in air. The crude reaction mixture was dissolved in H_2O/MeOH (2 mL, 5/1 v/v), charged on an RP column, and eluted with H_2O/MeCN (gradually from 20 to 60% of MeCN). Fractions containing the desired product **12** were evaporated, and the residue was dissolved in MeOH (1 mL), precipitated with Et_2O, centrifuged, and dried overnight under reduced pressure at 50 °C: yield 38 mg (92%); red powder, mp >190 °C dec; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.29−7.19 (m, 6H), 7.18−7.12 (m, 3H), 6.79 (d, 2H, J = 8.3 Hz), 6.61 (s, 1H), 6.25 (br s, 1H), 5.97 (s, 1H), 4.71 (d, 1H, J = 11.4 Hz), 4.60 (d, 1H, J = 8.0 Hz), 4.57−4.50 (m, 1H), 4.41−4.30 (m, 2H), 4.28−4.13 (m, 3H), 3.80−3.69 (m, 2H), 3.64 (d, 1H, J = 13.6 Hz), 3.59−3.54 (m, 1H), 3.43 (s, 1H), 3.21 (d, 1H, J = 10.5 Hz), 3.13 (dd, 1H, J = 5.7 and 13.9 Hz), 2.96−2.79 (m, 3H), 2.66 (s, 3H), 2.62−2.50 (m, 5H), 2.57 (s, 3H), 2.56 (s, 3H), 2.51−2.38 (m, 5H), 2.38−2.30 (m, 1H), 2.28 (s, 3H), 2.27 (s, 3H), 2.23−2.16 (m, 1H), 2.15−2.05 (m, 1H), 2.06−1.88 (m, 5H), 1.85 (s, 3H), 1.78−1.66 (m, 1H), 1.45 (s, 3H), 1.39−1.26 (m, 2H), 1.34 (s, 3H), 1.29 (s, 3H), 1.29 (s, 3H), 1.22 (s, 3H, J = 6.2 Hz), 1.10 (s, 3H), 0.50 (s, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 179.8, 178.2, 177.7, 177.5, 176.8, 176.0, 175.6, 175.0, 174.2, 173.9, 173.8, 172.1, 166.4, 165.8, 158.9, 143.6, 138.8, 138.6, 135.0, 133.3, 132.7, 131.9, 131.6, 130.2, 129.5, 128.2, 127.8, 121.0, 118.6, 112.1, 108.3, 104.9, 102.7, 95.3, 88.1, 86.2, 84.2, 83.7, 81.3, 79.5, 75.8, 75.3, 73.5, 70.5, 65.0, 59.9, 57.0, 56.7, 56.3, 55.1, 52.3, 46.5, 45.1, 44.1, 43.3, 39.9, 38.7, 36.4, 35.2, 33.3, 33.2, 32.7, 32.0, 29.4, 27.4, 26.4, 20.8, 20.4, 20.3, 20.2, 20.0, 17.5, 17.2, 16.5, 16.2; UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 551 (5.7×10^3), 522 (4.3×10^3), 364 (1.7×10^4), 262 (3.7×10^4), 218 (4.0×10^4); HRMS (ESI) m/z [M + 2Na]$^{2+}$ calcd for $C_{85}H_{108}N_{16}O_{17}PCoNa_2$ 880.3466, found 880.3464. Anal. Calcd for $C_{85}H_{108}N_{16}O_{17}PCo{\cdot}12H_2O$: C, 52.84; H, 6.89; N, 11.60. Found: C, 52.63; H, 7.11; N, 11.38. HPLC: t_R = 13.05 min.

Synthesis of Compound 14. CuI (1 mg, 0.005 mmol) and TBTA (5 mg, 0.01 mmol) were suspended in a DMF/H_2O mixture (0.7 mL, 3/1 v/v) and stirred until clear, a yellowish solution was formed. To this solution was added conjugate **12** (34 mg, 0.02 mmol) followed by addition of D-biotin-6-azido-*n*-hexylamide (**13**;[41] 9 mg, 0.02 mmol). The reaction mixture was stirred for 18 h at room temperature. The reaction workup and product purification were performed according to the procedure described for compound **3**: yield 33 mg (79%); red powder, mp >190 °C dec; ^{1}H NMR (CD_3OD, 500 MHz) δ 8.19 (s, 1H), 7.53 (d, 2H, J = 8.3 Hz), 7.29−7.10 (m, 7H), 6.91 (d, 2H, J = 8.3 Hz), 6.63 (s, 1H), 6.25 (br s, 1H), 5.98 (s, 1H), 4.71 (d, 1H, J = 11.1 Hz), 4.61 (d, 1H, J = 8.5 Hz), 4.57−4.49 (m, 1H), 4.47−4.33 (m, 5H), 4.30−4.13 (m, 4H), 3.79−3.69 (m, 2H), 3.64(d, 1H, J = 13.3 Hz), 3.55 (dd, 1H, J = 5.0 and 10.4 Hz), 3.22 (d, 1H, J = 11.0 Hz), 3.19−3.07 (m, 4H), 2.96−2.79 (m, 4H), 2.71−2.63 (m, 2H), 2.66 (s, 1H), 2.63−2.55 (m, 5H), 2.58 (s, 3H), 2.56 (s, 3H), 2.54−2.49 (m, 3H), 2.48−2.43 (m, 3H), 2.38−2.32 (m, 1H), 2.28 (s, 3H), 2.27 (s, 3H), 2.25−2.18 (m, 1H), 2.16 (t, 2H, J = 7.3 Hz), 2.12−1.96 (m, 4H), 1.95−1.88 (m, 4H), 1.88−1.80 (m, 2H), 1.86 (s, 3H), 1.78−1.51 (m, 5H), 1.51−1.42 (m, 2H), 1.45 (s, 3H), 1.42−1.28 (m, 5H), 1.35 (s, 6H), 1.30 (s, 3H), 1.22 (d, 3H, J = 6.2 Hz), 1.12 (s, 3H), 0.51 (s, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 179.8, 178.1, 177.7, 177.5, 176.8, 176.03, 175.96, 175.6, 175.0, 174.2, 173.9, 172.1, 166.4, 166.1, 165.7, 158.9, 148.4, 143.6, 138.6, 135.0, 133.3, 132.4, 131.6, 130.2, 129.5, 129.2, 127.8, 126.3, 122.0, 118.6, 108.4, 104.9, 95.3, 88.2, 86.2, 75.8, 73.4, 63.4, 61.6, 59.9, 57.0, 56.9, 56.3, 55.1, 52.4, 51.4, 48.2, 46.3, 45.1, 44.3, 43.6, 43.3, 41.2, 40.1, 39.9, 38.7, 36.8, 36.4, 35.2, 33.2, 32.8, 32.0, 31.1, 30.2, 29.7, 29.5, 27.4, 27.3, 27.1, 26.9, 26.4, 20.8, 20.5, 20.3, 20.2, 20.0, 17.5, 17.2, 16.5, 16.2; UV/vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 551 (5.2×10^3), 522 (4.5×10^3), 364 (1.8×10^4), 263 (2.5×10^4), 216 (2.7×10^4); HRMS (ESI) m/z [M + 2Na]$^{2+}$ calcd for $C_{101}H_{136}N_{22}O_{19}PCoSNa_2$ 1064.4463, found 1064.4447. Anal. Calcd for $C_{101}H_{136}N_{22}O_{19}PSCo{\cdot}12H_2O$: C, 52.73; H, 7.01; N, 13.40. Found: C, 52.37; H, 7.11; N, 13.03. HPLC: t_R = 12.95 min.

■ ASSOCIATED CONTENT

Supporting Information

Text, tables, and figures giving NMR spectra and HPLC chromatograms for all new products and computational details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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