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Microwave-Assisted Cobinamide Synthesis

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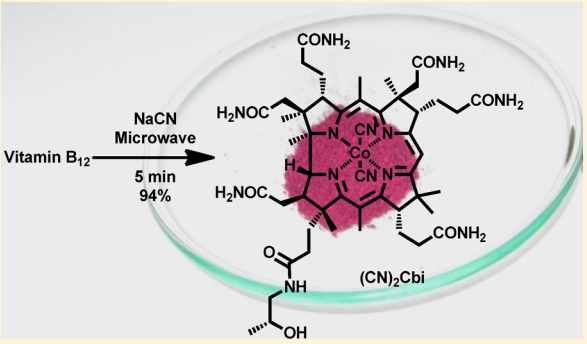
Supporting Information

ABSTRACT: We present a new method for the preparation of cobinamide $(CN)_2$Cbi, a vitamin B_{12} precursor, that should allow its broader utility. Treatment of vitamin B_{12} with only NaCN and heating in a microwave reactor affords $(CN)_2$Cbi as the sole product. The purification procedure was greatly simplified, allowing for easy isolation of the product in 94% yield. The use of microwave heating proved beneficial also for $(CN)_2$Cbi(*c*-lactone) synthesis. Treatment of $(CN)_2$Cbi with triethanolamine led to $(CN)_2$Cbi(*c*-lactam).



The use of cyanide as a poison dates back millennia; cyanide has been responsible for claiming countless lives.[1] Even now, cyanide poisoning is a real danger particularly in smoke inhalation, industrial accidents, and possible terrorist attacks. If a diagnosis of cyanide toxicity is strongly suspected, antidotes do exist, such as hydroxocobalamin (HO-Cbl), sodium nitrate, and sodium thiosulfate, but unfortunately, they must be administered intravenously and immediately, over a period of time, hindering their appeal when treating mass casualties.[2] Recently, Boss et al. reported cobinamide (Cbi), a vitamin B_{12} precursor, as a superior candidate for an antidote to cyanide poisoning and for its detection.[3] This is due to its higher affinity for cyanide, compared to that of the currently used (H_2O)Cbl [10^{12} M^{-1} compared to $\approx 10^{22}$ M^{-1} for (H_2O)(HO)Cbi].[4] Moreover, the effectiveness of Cbi increases when it is combined with sulfanegen, a known potent cyanide antidote that contrary to Cbl was found to be selective in -CN binding in the presence of -SCN.[5] Boss went on to create a disposable blood cyanide sensor. The blood was treated with an acid-expelling HCN gas, which was detected by Cbi.[6,7] On the other hand, Zelder utilized $(CN)(H_2O)$Cbi immobilized on silica for this purpose, allowing the detection of cyanide on a micromolar scale in blood, plants, and tobacco smoke.[8−10]

Cbi applications are not limited to cyanide poisoning; nitrosyl-cobinamide ((NO)Cbi) was reported to act as a novel NO donor for in *vitro* wound healing in several cell types and has shown improvement over known NO donors.[11] It has been also employed by our group for the activation of the sGC enzyme in a NO-independent manner, allowing vascular relaxation.[12]

Cbi is a vitamin B_{12} precursor having a corrin macrocycle bearing all six peripheral amide groups and an *iso*-propanolamide group at the *f*-position but lacks the nucleotide moiety of 5′,6′-dimethylbenzimidazole and ribose. Its synthesis from Cbl involves cleavage of the P−O bond, and even though there are established methods for the hydrolysis of phosphates, the complexity of the molecule makes it tremendously difficult. There are many examples detailing Cbi synthesis dating back to 1956 when Friedrich and Bernhaver obtained it in 60−80% yield by reacting Cbl with cerium(III) hydroxide.[13] Though the method seemed simple, the purification via consecutive phenol extractions and chromatography limits its broader use; nevertheless, it is still very popular. Treatment of (CN)Cbl with CF_3SO_3H under strictly anhydrous conditions gave (CN)Cbi in 91% yield at room temperature, but disadvantages included the unwelcoming anhydrous conditions.[14] The procedure stemmed from Hogenkamp's work in which he used these conditions in a nonanhydrous manner resulting in epimerization at position 8 giving CN-8-*epi*Cbl.[15] Surprisingly, treatment of expensive aquacobalamin with concentrated HCl afforded Cbi in 80% yield, though the method is more commonly known for conducting partial hydrolysis. No analysis or characterization of the isolated compound was reported.[16] In 1971, Bonnet discovered that the treatment of (CN)Cbl **1** with trifluoroacetic acid led to a mixture of products, from which cobinamide was purified.[17] Recently, Zelder et al. presented excellent work on Cbi synthesis.[18] $(CN)(H_2O)$Cbi was obtained in 63% yield by reacting (CN)Cbl **1** with either $Cu(NO_3)_2{\cdot}3H_2O$ or $ZnCl_2$ in MeOH. Even though a decrease in the yield was observed compared to that reported in Müller's work, who formed Cbi via treatment of (CN)Cbl with $ZnCl_2$ and NaCN, the purification process was much less demanding though still required preparative HPLC. A large scale Cbi preparation could not be performed, plateauing at 20 mg scale.[19] Keese also applied Müller's route by using hydrox-

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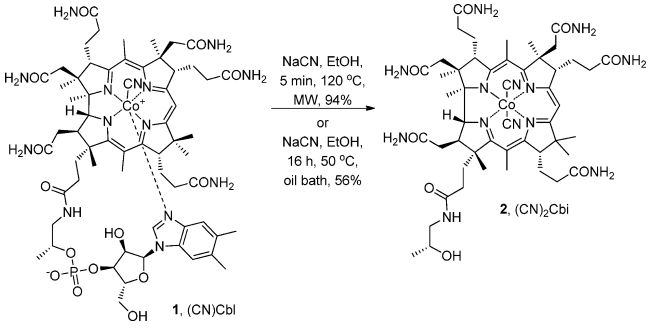
ocobalamin with $ZnCl_2$ in the presence of a reducing agent, giving Cbi in 56% yield.[20]

(CN)(H_2O)Cbi and (H_2O)(HO)Cbi hold promise as antidotes in cyanide poisoning and detection. Unfortunately, because of the unavailability of a simple and high-yield synthesis for the dicyano precursor $(CN)_2$Cbi **2** (Scheme 1), diaqua

Scheme 1. Synthesis of $(CN)_2$Cbi 2



complex is relatively expensive, which may preclude its broader use. If a new method is to be universally utilized, it should be simple and cheap with easy purification and isolation, while preferably being conducted in the absence of a metal catalyst due to possible future medical applications. Herein, we present the synthesis of $(CN)_2$Cbi adhering to these requirements.

The use of microwave reactors in organic synthesis has become an attractive tool, allowing full control over various parameters, such as temperature, pressure, etc., producing a positive outcome.[21] Utilizing microwave irradiation in vitamin B_{12} chemistry is relatively unheard of, the only reported studies being related to the decomposition of vitamin B_{12} in milk.[22,23] Because of the small quantity of decomposed products formed, their precise structure was not elucidated. However, the use of microwave-assisted synthesis in phosphate cleavage is known.[24] Our first attempt at cobinamide synthesis drew on the work of Müller, in which metal salts with NaCN were used.[19] Zelder excluded NaCN from this procedure; however, in our experience, we have found NaCN to be a very useful reagent in vitamin B_{12} chemistry.[18,25] Hence, we pondered the possibility of combining microwave irradiation with only NaCN. Subsequently, when (CN)Cbl **1** was reacted with an excess of NaCN (27 equiv) in EtOH at 120 °C for 5 min, we were pleased to find that the reaction proceeded in 95% conversion and furnished $(CN)_2$Cbi **2** in 94% isolated yield, the highest ever documented (Table 1, entry 1). The purification procedure involved a short normal phase silica column,

Table 1. Optimization of the Amount of NaCN in the Microwave-Assisted Synthesis of $(CN)_2$Cbi 2[a]

entry	(CN)Cbl (mmol)	NaCN (mmol) (equiv)	EtOH (mL)	$(CN)_2$Cbi (%)
1	0.01	0.30 (27)	1.0	94
2	0.01	0.15 (13)	1.0	90
3	0.01	0.075 (7.0)	1.0	80
4	0.01	0.037 (3.5)	1.0	65
5	0.01	0.037 (3.5)	0.1	93
6	0.07	0.25 (3.0)	6.6	92[b]

[a]The appropriate amounts of (CN)Cbl and NaCN were dissolved in EtOH and heated to 120 °C for 5 min in MW 300 W with stirring. [b]Reaction time of 10 min.

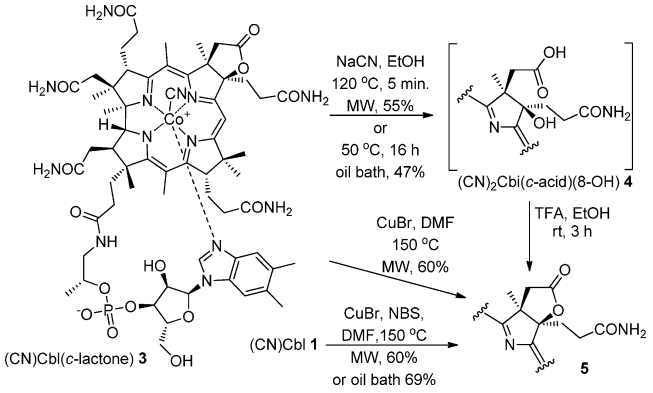
followed by filtration through a Celite plug to remove cyanide and silica. Surprisingly, the use of reverse phase column chromatography dramatically decreased the yield.

A brief optimization was conducted focusing on the amount of cyanide used because of its high toxicity (entries 2–6). Reactions conducted on a 0.011 mmol scale required only 3.5 equiv of NaCN, but a drop in yield to 65% was observed (entry 4). With an increase in the reaction concentration, the yield sprang back to a comfortable 93% (entry 5). Scale-up of the reaction to 0.07 mmol required a slight increase in time to 10 min, giving $(CN)_2$Cbi **2** in 92% isolated yield (entry 6). Other solvents examined produced little to no product. Notably, although undesirable, employing a great excess (27 equiv) of cyanide worked extremely well, the reaction being conducted on a 0.5 g (0.36 mmol) scale giving Cbi in 90% yield.

The use of a microwave reactor could be avoided though sacrificing the yield. The reaction was conducted in a Schlenck tube at 50 °C in an oil bath using 27 equiv of NaCN for 16 h and furnished $(CN)_2$Cbi **2** in 56% yield. This confirmed the universality of our method. In this case, an excess of NaCN is a must as a decrease in the amount of the promoter gave only traces of product. Please note that the use of a flask fitted with a septa or stopper gave inconsistent results. Furthermore, our method has the major advantage of mild reaction conditions.

Once the synthesis of $(CN)_2$Cbi **2** was accomplished, we decided to delve into its functionalization. To date, further reactions with $(CN)_2$Cbi **2** consisted only of coupling at the terminal hydroxyl group at the *f*-position.[26] Therefore, we examined the possibility of B-ring functionalization, commonly consisting of *c*-lactone formation. Methods normally involve treatment of Cbl with either chloramine-T or NBS.[27] We, therefore, took the newly synthesized cobinamide **2** and treated it with NBS under acidic conditions. Surprisingly, the reaction mixture turned brown, and a mixture of inseparable products was detected via HPLC. Alternatively, the order of steps was reversed by first using (CN)Cbl(*c*-lactone) **3** as a substrate in our newly developed procedure (Scheme 2). Only partial

Scheme 2. Synthesis of $(CN)_2$Cbi(*c*-lactone) 5



conversion was observed, highlighting the difference between vitamin B_{12} and its *c*-lactone derivative. An increase in the amount of NaCN back to 30 equiv gave full conversion. Unfortunately, NMR analysis of the reaction mixture was disappointing and indecipherable. MS showed only one peak at $[M - CN]^+$ 1032.47 correlating to $(CN)_2$Cbi(*c*-acid)(8-OH) **4** (Scheme 2). It was found that because of the presence of a base and H_2O, the lactone simply opened at a high temperature. From our previous work, we know that the presence of the C8-

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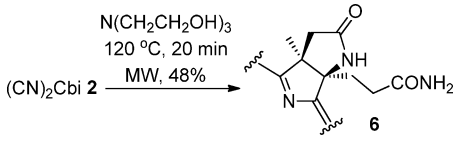
OH group makes the compound sensitive to acid.[25] Therefore, after NaCN removal, the crude reaction mixture was treated with 50% TFA in EtOH, giving the desired product, $(CN)_2$Cbi(*c*-lactone) **5**, in 55% yield, the first example of a B-ring-functionalized cobinamide derivative. Once again, the method could be easily transferred to the bench. When (CN)Cbl(*c*-lactone) **3** was treated with NaCN at 50 °C in an oil bath followed by exposure to an acid, $(CN)_2$Cbi(*c*-lactone) **5** was isolated in a comparable 47% yield.

Although the overall reaction worked very well, the elegance of the pathway demanded a direct route. Zelder detailed a promising reaction involving metal salts for Cbi synthesis.[18] We took this premise and employed CuBr. Fortunately, when (CN)Cbl(*c*-lactone) **3** was reacted with CuBr at 150 °C in the microwave reactor, $(CN)_2$Cbi(*c*-lactone) **5** was produced in 60% yield. Using $CuBr_2$ rendered the reaction inactive. To refine the method, (CN)Cbl **1** was dissolved in dry DMF and reacted with NBS and CuBr at 150 °C for 25 min. HPLC analysis of the crude mixture showed the presence of both (CN)Cbi **2** and (CN)Cbi(*c*-lactone) **5**, with the desired product being isolated in a pleasant 60% yield. This method was easily transferred to the bench, giving (CN)Cbi(*c*-lactone) **5** in 69% yield. ^{1}H NMR spectra of all synthesized (CN)Cbl(*c*-lactone) **5** were compared and showed almost identical results. Surprisingly, a doublet at ∼2.90 ppm varied in intensity throughout the spectra [please see Page S25 of the Supporting Information for a full comparison of all synthesized $(CN)_2$Cbi-(*c*-lactone) **5**]. This peak is not observed in the $(CN)_2$Cbi spectrum and does not correspond to the lactone moiety. In fact, it originates from the terminal -OH group at the *f*-position; because of a slow rate of exchange in CD_3OD, the intensity of the peak varies. A series of ^{1}H NMR spectra measured directly after dissolving and after 45 min and 24 h revealed that the peak disappears over time [for a full comparison of all synthesized (CN)Cbl(*c*-lactone) **5**, see Page S26 of the Supporting Information]. We postulated that this phenomenon occurred because of the presence of the lactone, allowing for interaction between the -OH and Co-CN groups. In our previous work, we have shown, via X-ray crystallography, that hydrogen bonding between a terminal -OH group and the Co-CN group is possible.[25]

Furthermore, treatment of (CN)Cbl **1** with NaOH furnishes the respective *c*-lactam.[28] We examined the premise that exposure to base causes lactam formation by treating $(CN)_2$Cbi **2** with triethanolamine at 120 °C for 20 min in the microwave, affording $(CN)_2$Cbi(*c*-lactam) **6** in 48% yield (Scheme 3).

Scheme 3. Synthesis of $(CN)_2$Cbi(*c*-lactam) 6



In conclusion, we have successfully synthesized $(CN)_2$Cbi **2** in excellent yield (94%) using microwave-assisted synthesis. The product was purified in the simplest possible way by using a combination of normal phase dry column vacuum chromatography (DCVC) and filtration with no preparative HPLC being required. This is the first example of Cbi synthesis in the microwave, in the shortest reaction time (5−10 min) and in the highest yield ever obtained. To make the method more universal, it was transferred to the bench. In a 50 °C oil bath, $(CN)_2$Cbi **2** was isolated in good yield. This is the mildest reaction condition ever used in a nonanaerobic way. Furthermore, the first examples of B-ring-functionalized Cbi derivatives were synthesized using our newly conceived method via the formation of *c*-lactone **3** and *c*-lactam **6**. Because of an inconvenient intermediate forming in the *c*-lactone reaction, a one-pot method was developed using CuBr. In all cases of *c*-lactone synthesis, the method was possible in a microwave reactor or in an oil bath.

EXPERIMENTAL SECTION

General. All solvents and chemicals used in the syntheses were of reagent grade and were used without further purification. Microwave-assisted synthesis was performed using microwave oven CEM Discover in sealed reaction vessels. The temperature was monitored using a vertically focused IR temperature sensor. UV−vis absorption spectra were measured at room temperature using a xenon lamp. ^{1}H and ^{13}C NMR spectra were recorded at rt on 500 and 400 MHz instruments with TMS as an internal standard. HRMS spectra were recorded on a spectrometer with a TOF mass analyzer. DCVC was performed using silica gel (200−300 mesh). Thin layer chromatography (TLC) was performed using silica gel GF254 (thickness of 0.20 mm). Reverse phase chromatography was performed using silica gel 90 C18. HPLC measurement conditions: column, Eurospher II 100-5 C18 250 mm × 4.6 mm with a precolumn; detection, UV−vis; wavelength, 361 nm; flow rate, 1 mL/min; pressure, 10 MPa; temperature, 30 °C (see the Supporting Information for gradient conditions).

*Synthesis of $(CN)_2$Cbi **2** Using NaCN in MW.* (CN)Cbl **1** (100 mg, 0.074 mmol) and NaCN (13 mg, 0.25 mmol) were added to a microwave reaction vessel equipped with a magnetic stirrer. EtOH (6.6 mL) was added, and the vessel was sealed with a cap. The vessel was heated to 120 °C for 10 min using 300 W. The resulting mixture was then transferred to a flask using EtOH, and double the volume of *i*PrOH was added. The solution was then loaded onto a normal phase silica column. Using MeOH in DCM (20−50%), the major violet fraction was isolated. The pure compound was dried *in vacuo*, redissolved in *i*PrOH, and filtered through a Ceilte plug to remove any excess cyanide and silica. The filtrate was then concentrated and recrystallized using an EtOH/Et_2O mixture, giving $(CN)_2$Cbi **2** as a purple solid in 92% (70 mg) yield: ^{1}H NMR (500 MHz, CD_3OD) δ 5.80 (s, 1H), 4.11 (d, J = 9.2 Hz, 1H), 3.83−3.78 (m, 2H), 3.55 (t, J = 6.7 Hz, 1H), 3.24−3.18 (m, 2H), 3.07 (ddd, J = 7.7, 6.4, 7.1 Hz, 1H), 2.98 (m, 1H), 2.63−2.56 (m, 3H), 2.53−2.46 (m, 2H), 2.42−2.36 (m, 2H), 2.34 (s, 3H), 2.32−2.28 (m, 1H), 2.27 (s, 5H), 2.25−2.20 (m, 2H), 2.17−2.02 (m, 6H), 1.99−1.79 (m, 4H), 1.69 (s, 3H), 1.51 (s, 6H), 1.42 (s, 3H), 1.28 (s, 3H), 1.91 (s, 3H), 1.13 (d, J = 6.4 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 178.36, 178.33, 178.2, 178.1, 177.6, 175.8, 175.8, 175.7, 174.97, 174.92, 174.97, 174.92, 173.8, 164.8, 164.5, 164.5, 106.2, 104.2, 92.1, 84.5, 76.5, 67.3, 60.1, 57.8, 56.7, 55.0, 50.5, 47.9, 47.8, 47.5, 44.8, 42.7, 40.3, 36.2, 34.1, 33.5, 32.9, 32.7, 32.6, 31.7, 28.4, 26.8, 26.6, 22.9, 20.9, 19.8, 19.4, 18.6, 17.3, 16.1; UV−vis (H_2O) λ (ε, M^{-1} cm^{-1}) 276 (9.86×10^3), 312 (7.16×10^3), 367 (1.83×10^4), 504 (4.47×10^3), 538 (6.18×10^3), 581 nm (5.91×10^3); HRMS (ESI) $[M - CN]^+$ calcd for $C_{49}H_{72}CoN_{12}O_8$ m/z 1015.4928, found m/z 1015.4928; HPLC t_R values of 17.3 and 20.9 min (Eurospher II 100-5 C18 250 mm × 4.6 mm column, MeCN/H_2O with 0.05% TFA, 1 mL/min). Anal. Calcd for $C_{50}H_{72}CoN_{13}O_8 \cdot 5H_2O$: C, 53.04; H, 7.30; N, 16.08. Found: C, 52.74; H, 7.15; N, 15.71.

*Large Scale Synthesis of $(CN)_2$Cbi **2** Using NaCN in MW.* (CN)Cbl **1** (500 mg, 0.36 mmol) and NaCN (100 mg, 2.0 mmol) were added to a microwave reaction vessel equipped with a magnetic stirrer. EtOH (33 mL) was added, and the vessel was sealed with a cap. The vessel was heated to 120 °C for 5 min using 300 W after which the reaction was worked up as per the first procedure using MW. **Caution:** *The large scale generates a very high pressure in the reactor.* $(CN)_2$Cbi **2** was isolated as a purple solid in 90% (365 mg) yield: ^{1}H NMR (500 MHz,

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CD_3OD) δ 5.80 (s, 1H), 4.11 (d, J = 8.3 Hz, 1H), 3.81 (m, 2H), 3.55 (t, J = 5.6 Hz, 1H), 3.24−3.18 (m, 2H), 3.07 (dd, J = 7.1, 6.6 Hz, 1H), 2.98 (m, 1H), 2.66−2.56 (m, 3H), 2.53−2.46 (m, 2H), 2.42−2.36 (m, 2H), 2.34 (s, 3H), 2.32−2.29 (m, 1H), 2.27 (s, 5H), 2.24−2.19 (m, 2H), 2.18−2.02 (m, 6H), 1.99−1.80 (m, 4H), 1.70 (s, 3H, C7a), 1.51 (s, 6H), 1.43 (s, 3H), 1.28 (s, 3H) 1.19 (s, 3H) 1.12 (d, J = 6.2 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 178.35, 178.31, 178.2, 178.15, 178.1, 177.6, 175.8, 175.7, 174.96, 174.91, 173.8, 164.8, 164.5, 106.1, 104.1, 92.1, 84.5, 76.5, 67.3, 60.0, 57.8, 56.7, 55.0, 50.5, 47.9, 47.8, 47.4, 44.8, 42.7, 40.3, 36.2,34.1, 33.5, 32.9, 32.7, 32.6, 31.7, 28.4, 26.8, 26.6, 22.9, 20.9, 19.8, 19.4, 18.3, 17.3, 16.1, 15.8 (for the full assignment of ^{1}H and ^{13}C spectra, see the Supporting Information); LRMS (ESI) $[M + Na]^+$ calcd for $C_{50}H_{72}CoN_{13}O_8Na$ m/z 1064.48, found m/z 1064.49; HPLC t_R values of 17.2 and 20.7 min (Eurospher II 100-5 C18 250 mm × 4.6 mm column, MeCN/H_2O with 0.05% TFA, 1 mL/min).

Synthesis of (CN)$_2$Cbi ***2*** *Using NaCN in an Oil Bath.* (CN)Cbl **1** (50 mg, 0.037 mmol) and NaCN (50 mg, 1.0 mmol) were added to a 10 mL Schlenk tube, and EtOH (3.3 mL) was added. The reaction mixture was placed in a preheated oil bath at 50 °C for 16 h, after which the reaction was worked up as per the procedure using MW. $(CN)_2$Cbi **2** was isolated as a purple solid in 56% (22 mg) yield: ^{1}H NMR (500 MHz, CD_3OD) δ 5.80 (s, 1H), 4.10 (d, J = 9.6 Hz, 1H), 3.83−3.78 (m, 2H), 3.55 (t, J = 6.0 Hz, 1H), 3.24−3.18 (m, 2H), 3.08−3.04 (m, 1H), 3.00−2.96 (m, 1H), 2.63−2.56 (m, 3H), 2.52−2.46 (m, 2H), 2.42−2.36 (m, 2H), 2.34 (bs, 4H), 2.27 (s, 5H), 2.24−2.19 (m, 2H), 2.18−2.02 (m, 6H), 1.99−1.81 (m, 4H), 1.70 (s, 3H), 1.51 (s, 6H), 1.43 (s, 3H), 1.28 (s, 3H), 1.19 (s, 3H), 1.13 (d, J = 6.5 Hz, 3H); LRMS (ESI) $[M + Na]^+$ calcd for $C_{50}H_{72}CoN_{13}O_8Na$ m/z 1064.49, found m/z 1064.49; HPLC t_R values of 17.2 and 20.7 min (Eurospher II 100-5 C18 250 mm × 4.6 mm column, MeCN/H_2O with 0.05% TFA, 1 mL/min).

(CN)Cbl(c-lactone) ***3.*** (CN)Cbl(c-lactone) **3** was synthesized using a modified procedure of Keese.[27] (CN)Cbl **1** (500 mg, 0.36 mmol) and NBS (115 mg, 0.65 mmol) were dissolved in H_2O (150 mL) and treated with AcOH (7.5 mL), and then the mixture was stirred for 24 h at room temperature. The reaction mixture was transferred to a separating funnel, washed with CH_2Cl_2, and then evaporated to dryness. The residue was then recrystallized using a MeOH/Et_2O mixture giving (CN)Cbl(c-lactone) **3** (494 mg, 99%) as a purple solid in 86% purity. **3** was used for further reactions without any purification. For analytical purposes, (CN)Cbl(c-lactone) was purified using RP column chromatography, with a 5% CH_3CN/H_2O mixture, isolating the first major fraction. The isolated product was evaporated to dryness and recrystallized using a MeOH/Et_2O mixture, giving (CN)Cbl(c-lactone) **3** as a purple solid in 61% (305 mg) yield in 99% purity: ^{1}H NMR (500 MHz, CD_3OD) δ 7.29 (s, 1H), 7.16 (s, 1H), 6.49 (s, 1H), 6.28 (s, 1H), 5.99 (s, 1H), 4.45 (d, J = 8.4 Hz, 1H), 4.43−4.20 (m, 2H), 4.13 (d, J = 12.1 Hz, 2H), 4.09−3.76 (m, 3H), 3.67 (d, J = 12.1 Hz, 1H), 3.18 (d, J = 17.7 Hz, 1H), 2.90 (m, 1H), 2.84 (d, J = 19.1 Hz, 2H), 2.61 (m, 11H), 2.53−2.35 (m, 8H), 2.27 (m, 8H), 2.15 (m, 1H), 2.05 (m, 1H), 1.92 (s, 4H), 1.86−1.80 (m, 3H), 1.59 (t, J = 11.2 Hz, 1H), 1.49 (s, 3H), 1.39 (s, 3H), 1.35 (s, 3H), 1.29 (s, 3H), 1.16 (m, 3H), 0.46 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 181.7, 180.9, 180.0, 174.3, 169.3, 166.8, 163.4, 143.4, 138.3, 135.8, 134.1, 131.5, 117.2, 112.9, 108.2, 106.8, 95.4, 92.5, 88.0, 86.5, 77.0, 70.7, 60.5, 57.8, 55.0, 53.4, 53.3, 50.0, 46.8, 52.9, 42.1, 40.1, 36.0, 35.2, 33.4, 32.9, 32.2, 31.9, 30.4, 29.5, 27.3, 20.9, 20.6, 20.3, 20.2, 20.1, 20.0, 17.4, 17.3, 17.1, 16.2; UV−vis (H_2O) λ (ε, M^{-1} cm^{-1}) 206 (4.98×10^3), 276 (4.34×10^3), 359 (4.54×10^3), 522 (4.04×10^3), 540 nm (4.04×10^3); HRMS (ESI) $[M + Na]^+$ calcd for $C_{63}H_{85}CoN_{13}O_{15}PNa$ m/z 1376.5240, found m/z 1376.5255. HPLC t_R value of 14.9 min (Eurospher II 100-5 C18 250 mm × 4.6 mm column, MeCN/H_2O with 0.05% TFA, 1 mL/min). Anal. Calcd for $C_{63}H_{85}CoN_{13}O_{15}P{\cdot}3H_2O$: C, 53.73; H, 6.51; N, 12.93. Found: C, 53.87; H, 6.43; N, 12.78.

Synthesis of (CN)$_2$Cbi(c-lactone) ***5*** *Using NaCN in MW.* (CN)-Cbl(c-lactone) **3** (40 mg, 0.03 mmol) and NaCN (40 mg, 0.8 mmol) were placed into a microwave reaction vessel and dissolved in EtOH (2.6 mL), and the vessel was sealed with a cap. The mixture was heated in a microwave reactor for 5 min at 120 °C using 300 W with stirring. It was then transferred to a round-bottom flask and the solvent evaporated to dryness. The residue was redissolved in iPrOH and passed through a Ceilte plug. The filtrate was evaporated to dryness, then dissolved using a 50% TFA/EtOH mixture, and allowed to stir at room temperature for 3 h. The solvent was then removed, redissolved in iPrOH, and placed onto a DCVC column with NaCN. Using MeOH in DCM (20−50%), the major violet fraction was isolated. The pure compound was dried *in vacuo*, redissolved in iPrOH, and filtered through a Ceilte plug to remove any excess cyanide and silica. The filtrate was then concentrated and recrystallized using an EtOH/Et_2O mixture, giving $(CN)_2$Cbi(c-lactone) **5** as a purple solid in 55% (17 mg) yield: ^{1}H NMR (600 MHz, CD_3OD) δ 5.78 (s, 1H), 4.10 (d, J = 7.8 Hz, 1H), 3.82−3.79 (m, 2H), 3.24−3.20 (m, 2H), 3.09−3.01 (m, 3H), 2.66−2.58 (m, 3H), 2.52−2.39 (m, 4H), 2.37 (s, 4H), 2.35−2.29 (m, 1H), 2.27 (s, 5H), 2.25−2.20 (m, 2H), 2.16−2.10 (m, 3H), 2.06−1.82 (m, 5H), 1.71 (s, 3H), 1.52 (s, 3H), 1.51 (s, 3H), 1.44 (s, 3H), 1.28 (s, 3H), 1.17 (s, 3H), 1.11 (d, J = 5.7 Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 179.0, 177.6, 176.78, 176.73, 176.1, 175.0, 174.3, 174.2, 173.3, 173.29, 173.27, 165.8, 162.7, 160.4, 104.7, 104.4, 94.5, 87.6, 83.3, 75.6, 65.4, 56.4, 53.6, 50.2, 46.5, 46.1, 41.2, 38.8, 34.7, 32.9, 32.0, 31.4, 31.2, 29.7, 29.6, 29.0, 25.7, 25.1, 21.5, 19.5, 18.4, 18.3, 17.1, 14.5, 14.0; UV−vis (H_2O) λ (ε, M^{-1} cm^{-1}) 277 (9.21×10^3), 320 (8.35×10^3), 354 (2.02×10^4), 405 (3.78×10^3), 501 (7.09×10^3), 528 nm (7.13×10^3); HRMS (ESI) $[M + Na]^+$ calcd for $C_{50}H_{69}CoN_{12}O_9Na$ m/z 1063.4513, found m/z 1063.4540; HPLC t_R values of 18.6 and 22.1 min (Eurospher II 100-5 C18 250 mm × 4.6 mm column, MeCN/H_2O with 0.05% TFA, 1 mL/min).

Synthesis of (CN)$_2$Cbi(c-lactone) ***5*** *Using NaCN in an Oil Bath.* (CN)Cbl(c-lactone) **3** (40 mg, 0.03 mmol) and NaCN (40 mg, 0.8 mmol) were placed into Schlenk tube and dissolved in EtOH (2.6 mL). The reaction mixture was placed into a preheated oil bath at 50 °C for 16 h, after which the reaction was continued and worked up as per the procedure using MW. $(CN)_2$Cbi(c-lactone) **5** was isolated as a purple solid in 60% (14 mg) yield: ^{1}H NMR (500 MHz, CD_3OD) δ 5.78 (s, 1H), 4.08 (d, J = 8.3 Hz, 1H), 3.82 (m, 2H), 3.24−3.21 (m, 2H), 3.10−3.01 (m, 3H), 2.88 (d, J = 20.5 Hz, 1H), 2.64−2.58 (m, 3H), 2.50−2.40 (m, 4H), 2.37 (s, 4H), 2.35−2.30 (m, 1H), 2.28 (s, 5H), 2.25−2.22 (m, 2H), 2.16−2.10 (m, 3H), 2.08−1.83 (m, 5H), 1.72 (s, 3H), 1.53 (s, 3H), 1.52 (s, 3H), 1.45 (s, 3H), 1.28 (s, 3H), 1.18 (s, 3H), 1.13 (d, J = 5.8 Hz, 3H); HRMS (ESI) $[M + Na]^+$ calcd for $C_{50}H_{69}CoN_{12}O_9Na$ m/z 1063.4523, found m/z 1063.4540; HPLC t_R values of 18.5 and 21.9 min (Eurospher II 100-5 C18 250 mm × 4.6 mm column, MeCN/H_2O with 0.05% TFA, 1 mL/min).

Synthesis of (CN)$_2$Cbi(c-lactone) ***5*** *from (CN)Cbl(c-lactone)* ***3*** *Using CuBr in MW.* (CN)Cbl(c-lactone) **3** (31 mg, 0.02 mmol) and CuBr (35 mg, 0.2 mmol) were placed into the microwave reaction vessel and dissolved in DMF (0.8 mL), and the vessel was sealed with a cap. The mixture was heated in a microwave reactor for 25 min at 150 °C using 300 W with stirring. The reaction mixture was diluted with EtOH and transferred to a flask to which Et_2O was added. The precipitate was filtered through a cotton plug and then washed through with MeOH. The MeOH filtrate was then concentrated and the crude product purified using DCVC (20−50%) in MeOH in DCM. The pure compound was dried *in vacuo*, redissolved in iPrOH, and filtered through a Ceilte plug to remove any silica. The filtrate was then concentrated and recrystallized using an EtOH/Et_2O mixture, giving $(CN)_2$Cbi(c-lactone) **5** as a purple solid in 60% (14 mg) yield: ^{1}H NMR (400 MHz, CD_3OD) δ 5.78 (s, 1H), 4.09 (d, J = 8.6 Hz, 1H), 3.83−3.79 (m, 2H), 3.25−3.20 (m, 2H), 3.11−3.00 (m, 3H), 2.88 (d, J = 18.6 Hz, 1H), 2.68−2.57 (m, 3H), 2.50−2.40 (m, 4H), 2.37 (s, 4H), 2.35−2.30 (m, 1H), 2.28 (s, 5H), 2.25−2.18 (m, 2H), 2.16−2.10 (m, 3H), 2.05−1.85 (m, 5H), 1.72 (s, 3H), 1.52 (s, 6H), 1.45 (s, 3H), 1.28 (s, 3H), 1.18 (s, 3H), 1.12 (d, J = 6.1 Hz, 3H); HRMS (ESI) $[M - CN]^+$ calcd for $C_{49}H_{69}CoN_{11}O_9$ m/z 1014.4611, found m/z 1014.4612; HPLC t_R values of 18.3 and 20.0 min (Eurospher II 100-5 C18 250 mm × 4.6 mm column, MeCN/H_2O with 0.05% TFA, 1 mL/min).

Synthesis of (CN)$_2$Cbi(c-lactone) ***5*** *from (CN)Cbl* ***1*** *Using CuBr and NBS in MW.* (CN)Cbl **1** (30 mg, 0.02 mmol), NBS (4.2 mg, 0.02

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mmol), and CuBr (34 mg, 0.2 mmol) were placed in a microwave reaction vessel and dissolved in DMF (0.8 mL), and the vessel was sealed with a cap. The mixture was heated in a microwave reactor for 25 min at 150 °C using 300 W with stirring. $(CN)_2$Cbi(*c*-lactone) **5** was isolated as a purple solid in 60% (14 mg) yield: ^{1}H NMR (500 MHz, CD_3OD) δ 5.78 (s, 1H), 4.08 (d, J = 7.8 Hz, 1H), 3.83−3.79 (m, 2H), 3.24−3.20 (m, 2H), 3.10−3.01 (m, 3H), 2.89 (d, J = 18.6 Hz, 1H), 2.63−2.59 (m, 3H), 2.50−2.38 (m, 4H), 2.37 (s, 4H), 2.35−2.30 (m, 1H), 2.28 (s, 5H), 2.25−2.20 (m, 2H), 2.14−2.08 (m, 3H), 2.05−1.84 (m, 5H), 1.72 (s, 3H), 1.53 (s, 3H), 1.52 (s, 3H), 1.45 (s, 3H), 1.28 (s, 3H), 1.18 (s, 3H), 1.13 (d, J = 6.4 Hz, 3H); HRMS (ESI) $[M + Na]^+$ calcd for $C_{50}H_{69}CoN_{12}O_9Na$ m/z 1063.4500, found m/z 1063.4540; HPLC t_R values of 17.1 and 20.7 min (Eurospher II 100-5 C18 250 mm × 4.6 mm column, MeCN/H_2O with 0.05% TFA, 1 mL/min).

*Synthesis of $(CN)_2$Cbi(c-lactone) **5** from (CN)Cbl **1** Using CuBr and NBS in an Oil Bath.* (CN)Cbl **1** (31 mg, 0.02 mmol), NBS (4.5 mg, 0.2 mmol), and CuBr (33 mg, 0.2 mmol) were placed in a Schlenk tube and dissolved in DMF (0.8 mL). The reaction mixture was placed into a preheated oil bath at 150 °C for 16 h, after which the reaction continued and was worked up as per the procedure using MW. $(CN)_2$Cbi(*c*-lactone) **5** was isolated as a purple solid in 69% (17 mg) yield: ^{1}H NMR (500 MHz, CD_3OD) δ 5.78 (s, 1H), 4.08 (d, J = 8.6 Hz, 1H), 3.83−3.79 (m, 2H), 3.24−3.20 (m, 2H), 3.10−3.01 (m, 3H), 2.88 (d, J = 18.5 Hz, 1H), 2.70−2.58 (m, 3H), 2.53−2.39 (m, 4H), 2.37 (s, 4H), 2.35−2.30 (m, 1H), 2.28 (s, 5H), 2.25−2.20 (m, 2H), 2.16−2.10 (m, 3H), 2.08−1.84 (m, 5H), 1.72 (s, 3H), 1.53 (s, 3H), 1.52 (s, 3H), 1.45 (s, 3H), 1.28 (s, 3H), 1.18 (s, 3H), 1.13 (d, J = 6.4 Hz, 3H); HRMS (ESI) $[M + Na]^+$ calcd for $C_{50}H_{69}CoN_{12}O_9Na$ m/z 1063.4548, found m/z 1063.4540. HPLC t_R values of 18.5 and 19.5 min (Eurospher II 100-5 C18 250 mm × 4.6 mm column, MeCN/H_2O with 0.05% TFA, 1 mL/min).

*$(CN)_2$Cbi(c-lactam) **6**.* $(CN)_2$Cbi **2** (30 mg, 0.03 mmol) was placed in a microwave reaction vessel, and triethanolamine (3 mL) was added and the vessel sealed with a cap. The mixture was heated in a microwave reactor for 20 min at 120 °C using 300 W with stirring. It was then diluted with EtOH, and Et_2O was added until a compound began to precipitate. The solid was filtered through a cotton plug and the filtrate discarded. NaCN was added on top of the cotton, and the product was washed through using MeOH into a round-bottom flask. The solution was then evaporated to almost dry; *i*PrOH was added and the mixture loaded onto a DCVC column. Using MeOH in DCM (20−50%), the major violet fraction was isolated. The pure compound was dried *in vacuo*, redissolved in *i*PrOH, and filtered through a Ceilte plug to remove any excess cyanide and silica. The filtrate was then concentrated and recrystallized using an EtOH/Et_2O mixture giving $(CN)_2$Cbi(*c*-lactone) **6** as a purple solid in 48% (14 mg) yield: ^{1}H NMR (500 MHz, CD_3OD) δ 5.82 (s, 1H), 4.06 (d, J = 9.9 Hz, 1H), 3.84−3.78 (m, 2H), 3.25−3.20 (m, 2H), 3.10−3.00 (m, 2H), 2.71−2.69 (d, J = 9.4 Hz, 2H), 2.64−2.58 (m, 3H), 2.54−2.38 (m, 4H), 2.36 (s, 3H), 2.34−2.30 (m, 1H), 2.27 (s, 4H), 2.25−2.19 (m, 3H), 2.13−2.04 (m, 4H), 2.03−1.83 (m, 4H), 1.68 (s, 3H), 1.53 (s, 6H), 1.43 (s, 3H), 1.27 (s, 3H), 1.18 (s, 3H), 1.12 (d, J = 7.9 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 180.1, 178.8, 178.1, 178.0, 177.5, 176.9, 176.2, 175.7, 174.8, 171.5, 164.3, 163.3, 105.4, 105.3, 88.8, 84.6, 77.0, 75.7, 67.3, 60.1, 57.9, 55.1, 51.8, 47.9, 47.4, 46.3, 42.6, 40.2, 36.2, 34.4, 33.5, 32.8, 32.7, 31.8, 31.5, 31.2, 30.9, 27.1, 26.5, 22.9, 20.9, 20.4, 19.8, 18.6, 17.2, 17.0; UV−vis (H_2O) λ (ε, M^{-1} cm^{-1}) 275 (6.97×10^3), 311 (6.36×10^3), 364 (1.79×10^4), 507 (4.49×10^3), 540 (6.42×10^3), 580 nm (5.31×10^3); HRMS (ESI) $[M + Na]^+$ calcd for $C_{50}H_{70}CoN_{13}O_8Na$ m/z 1062.4695, found m/z 1062.4678. HPLC t_R values of 16.2 and 18.8 min (Eurospher II 100-5 C18 250 mm × 4.6 mm column, MeCN/H_2O with 0.05% TFA, 1 mL/min). Anal. Calcd for $C_{50}H_{70}CoN_{13}O_8 \cdot 5H_2O$: C, 53.14; H, 7.13; N, 16.11. Found: C, 53.19; H, 6.77; N, 15.92.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

All experimental procedure details and complete analytical data for new products. 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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