Efficient and Convenient Method for Axial Nucleotide Removal from Vitamin B_{12} and Its Derivatives

Xiang Zou, Daniel R. Evans, and Kenneth L. Brown*

Department of Chemistry, Mississippi State University, Drawer CH, Mississippi State, Mississippi 39762

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Cobinamides are intermediates in the biosynthesis[1] of vitamin B_{12} (cyanocobalamin, CNCbl)[2] and important compounds for studying the chemistry and biochemistry of B_{12}-requiring reactions.[3] Cobinamide derivatives are usually prepared from cyanocobinamide (factor B),[4] CNCbi, which is obtained chemically by semisynthesis from the readily available CNCbl. This involves selective cleavage of the phosphodiester bond of the nucleotide loop to remove the axial α-D-ribofuranosyl-5,6-dimethylbenzimidazole (α-ribazole)[2] in CNCbl while leaving the amide functional groups unaltered (Figure 1). Due largely to their negative charge, unactivated phosphodiesters are generally much less reactive than amides toward hydrolytic cleavage.[5] Thus, simple acid or base hydrolysis of CNCbl unavoidably leads to a mixture of factor B and corrinoid carboxylic acid derivatives, the latter resulting from the hydrolysis of some or all of the seven peripheral amides in the molecule.[6]

Currently, the most commonly used method for the removal of the axial nucleotide from cobalamin compounds employs an aqueous suspension of cerous(III) hydroxide gel in the presence of excess cyanide, which cleaves the phosphodiester bond of base-off dicyanocobalamin (formed in excess cyanide) in reasonable yield (usually 60−80%).[7] However, this procedure requires reaction at high temperature (1 h at 100 °C), which precludes its use for thermally labile compounds. In addition, it involves several time-consuming steps, including preparation of the cerous hydroxide gel with repeated washing and centrifugation, preparation of a toxic HCN solution by ion exchange chromatography, and several phenol extractions and column chromatographies—typically a full week's project for an experienced worker. An alternative method[8] utilizes corrosive hydrofluoric acid and thus has seldom been used. Attempts to use a phosphodiester-cleaving reagent, [Co(tris(3-aminopropyl)amine)$(H_2O)_2$]$^{3+}$, have not been successful.[9] Here, we report a simple, efficient, and nearly quantitative method for nucleotide removal from vitamin B_{12} derivatives at room temperature using trifluoromethanesulfonic acid (CF_3SO_3H).

In a typical experiment, cyanocobalamin (50 mg, 0.037 mmol), dried at room temperature over P_2O_5 under vacuum for 12 h,[10] was dissolved in 1 g (0.6 mL) of anhydrous CF_3SO_3H (Aldrich) under N_2.[11] The resulting solution was stirred for 24 h at room temperature, and the reaction mixture was poured into a sodium phosphate solution (100 mL, 1 M, dibasic form, pH 9.2), containing a trace amount of KCN (0.1 mg, 0.015 mmol). The solution was loaded onto an Amberlite XAD-2 column, and after thorough washing with water and 4% aqueous acetonitrile to remove the nucleotide byproducts, CNCbi was eluted with 50% aqueous acetonitrile.[4,12] The solvent was removed by evaporation, and the total yield of CNCbi was 91%. HPLC analysis showed that the product was free of nucleotide byproducts and of starting material.[13] The identity of the product was confirmed by its ^1H and ^{13}C NMR spectra[15] in an excess of potassium cyanide, its UV−visible spectrum, and HPLC coinjection with an authentic sample prepared by the $Ce(OH)_3$ method.[7a,d] A separate reaction starting with 500 mg of CNCbl and 10 g of CF_3SO_3H produced factor B in 85% yield.

This method is potentially useful for thermally labile vitamin B_{12} derivatives since it does not require heating. Cyano-8-epicobalamin, CN-8-epiCbl,[2,16] is a heat-sensitive vitamin B_{12} diastereomer, in which epimerization at corrin ring C8 has placed the *d* propionamide side chain in a pseudoequatorial position (Figure 1). This compound rapidly epimerizes back to CNCbl above 60 °C.[16b] When cerous(III) hydroxide was used to cleave the nucleotide loop from CN-8-epiCbl, no CN-8-epiCbi was obtained. Instead, the product was found to be factor B; i.e., in addition to cleavage of the nucleotide loop, the *d* side chain was epimerized to its natural configuration

* To whom correspondence should be addressed.

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(2) Abbreviations: Cbl = cobalamin; Cbi = cobinamide; CN-13-epiCbi = cyano-13-epicobinamide, in which the configuration at the corrin ring C13 is inverted, causing the *e* propionamide side chain to become "upwardly" axial; CN-8-epiCbi = cyano-8-epicobinamide, in which the configuration of the *d* side chain at C8 is likewise inverted; α-ribazole = α-D-ribofuranosyl-5,6-dimethylbenzimidazole; β-RCbi = β-alkylcobinamide; β-RCbl = β-alkylcobalamin; α-RCbi = α-alkylcobinamide.

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(4) Factor B (here abbreviated as CNCbi)[2] is a mixture of the diastereomeric α-(CN)-β-(H_2O)-Cbi and α-(H_2O)-β-(CN)-Cbi, in which the cyanide ligand occupies the "lower" and "upper" axial ligand positions, respectively. The structure is shown in Figure 1.

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(10) Excessive drying at high temperatures over P_2O_5 is not necessary. Separate cleavage reactions using three samples of CNCbl, which were dried by different methods, did not show any difference in yield ((1) commercial crystalline CNCbl dried over P_2O_5 at 95 °C under vacuum overnight in an Abderhälden apparatus; (2) commercial crystalline CNCbl dried over P_2O_5 at room temperature under vacuum overnight in a desiccator; (3) an aqueous solution of CNCbl evaporated to dryness under vacuum, redissolved in methanol, evaporated to dryness, and finally dried over P_2O_5 at room temperature under vacuum overnight in a desiccator).

(11) The mixing was carried out in a glovebox or a nitrogen glovebag.

(12) Some factor B (about 10%) was eluted with the nucleotide during the initial washing with 4% aqueous acetonitrile due to column "bleeding". This was recovered by a second chromatography on an XAD-2 column. Experiments involving acetonitrile should be carried out in a fumehood to avoid hazardous vapor.

(13) The purity of the product was over 93% (by HPLC integration at 254 nm), adequate for most purposes. High-purity samples can be obtained by further column chromatography[7d] or by semipreparative HPLC.[14]

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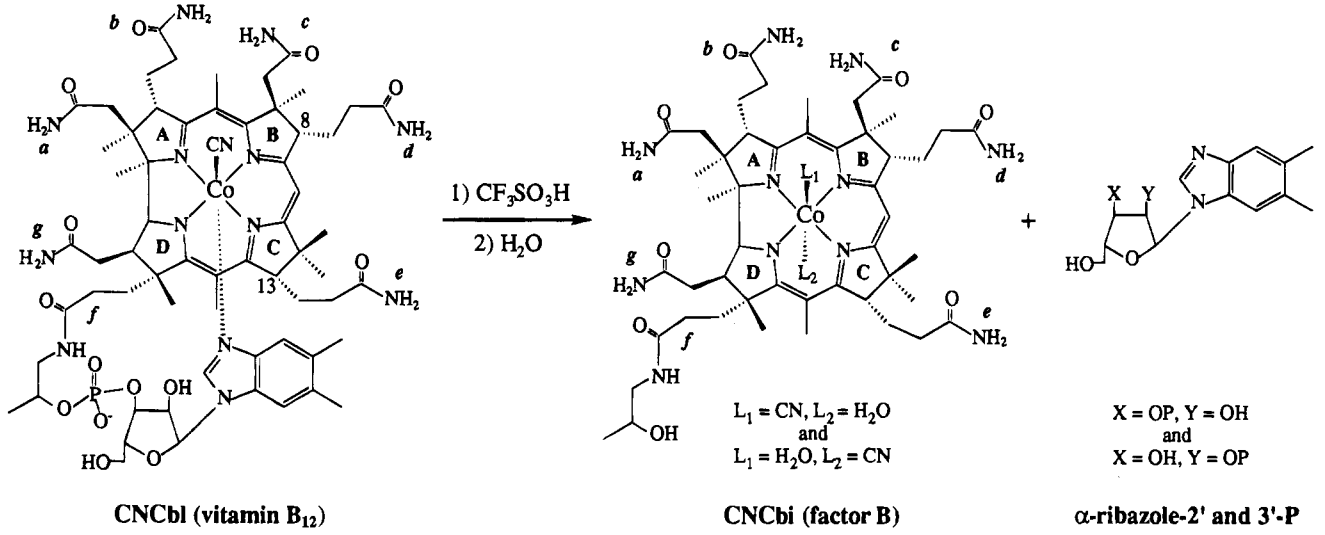

Figure 1. Structures of CNCbl and factor B and the phosphodiester cleavage CNCbl by CF_3SO_3H.

because of the high temperature. On the other hand, the CF_3SO_3H method gave CN-8-epiCbi at ambient temperature in virtually quantitative yield without detectable epimerization.[17]

Organocobinamides are generally synthesized by reductive alkylation of CNCbi with alkyl halides. Such reactions are now known to produce mixtures of diastereomeric products in which the organic ligand is in the "upper" (β) or "lower" (α) axial ligand position, the ratio of the two diastereomers depending strongly on the alkyl group.[18] For some alkyl groups, such as CF_3- and CF_2H-, direct reductive alkylation of CNCbi gives >90% of the α-isomer.[18b] Thus, direct synthesis of β-RCbi's from β-RCbl's by nucleotide loop cleavage at room temperature without substantial decomposition of the alkyl products would be quite useful since β-RCbl's can usually be prepared easily by direct alkylation. The new method reported here serves this need. When β-CF_2HCbl was reacted with CF_3SO_3H at room temperature, α-ribazole was cleanly removed to give a 78% yield of β-CF_2HCbi. In contrast, reductive alkylation of CNCbi gives only ~2% of the β diastereomer.[18b]

Hogenkamp and co-workers[19] previously reported the CF_3SO_3H-catalyzed epimerization of cyanocobalamin-*e*-monocarboxylate to cyano-13-epicobalamin-*e*-monocarboxylate, in which the configuration at C13 is inverted.[2] However, no epimerization at C13 was detected in any of the preparations reported here using anhydrous conditions. Instead, the current study shows that CF_3SO_3H effects the selective cleavage of the phosphodiester bond in a variety of vitamin B_{12} derivatives at room temperature. When H_2O (10% v/v) was added to CF_3SO_3H, its reaction with CNCbl for 24 h led to two products, CNCbi (65%) and CN-13-epiCbi (26%). These results show that strictly anhydrous conditions are necessary if CN-13-epiCbi is not the desired product. The efficiency of CF_3SO_3H in the hydrolysis reaction appears to be due to its strong acidity when it serves as both a reagent and the solvent. Under these strongly acidic conditions, the phosphodiester in CNCbl is fully protonated and thus resembles a phosphotriester in reactivity, making it much more reactive toward cleavage than the side chain amides and a normal phosphodiester anion.[5,20] The byproducts of the cleavage, the residue of the nucleotide loop, were found to be α-ribazole 2'- and 3'-phosphate.[2] In the HPLC chromatogram, they were clearly seen as two distinct peaks that had UV spectra identical with that of α-ribazole[7d] and were eluted slightly earlier than CNCbi. These results show that the cleavage probably occurs via intramolecular, nucleophilic attack on the phosphodiester by the 2'-hydroxyl of the nucleotide, forming initially α-ribazole 2',3'-cyclic phosphate[21] and CNCbi. Upon addition of water, the cyclic nucleotide undergoes hydrolysis to give a mixture of the 2'- and 3'-nucleotides.[22]

In conclusion, cleavage of the phosphodiester in vitamin B_{12} and its derivatives can be readily accomplished using neat CF_3SO_3H under anhydrous conditions at room temperature. In addition to the high yield, the new method is much more convenient and less time-consuming than the current cerous hydroxide method.[7] As demonstrated by the synthesis of β-CF_2HCbi and CN-8-epiCbi, this method is also useful for high-yield, stereospecific synthesis of β-RCbi's and CNCbl derivatives by phosphodiester cleavage of their corresponding cobalamin compounds, either when these complexes are thermally labile or where direct alkylation of cobinamide gives rise primarily to the α-RCbi's.[18]

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Supplementary Material Available: A table of tentative ^{13}C NMR chemical shift assignments for $(CN)_2$-8-epiCbi and comparison with those of $(CN)_2$Cbi and CN-8-epiCbl and a diagram showing the numbering scheme used in the assignments (3 pages). Ordering information is given on any current masthead page.

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(17) Like factor B,[2] CN-8-epiCbi is also a mixture of the diastereomeric α-(CN)-β-(H_2O)-8-epiCbi and α-(H_2O)-β-(CN)-8-epiCbi. It was characterized by its ^{13}C NMR spectrum as the dicyano derivative, which was assigned by analogy to that of CN-8-epiCbl[16b] and $(CN)_2$Cbi.[15] These assignments are given as supplementary material.

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(22) These nucleotides can be recovered by evaporation of the first fraction of the acetonitrile eluents and separated by HPLC.