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Vitamin B₁₂ and α -ribonucleosides

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Abbreviations: B₁₂, vitamin B₁₂; CNCbl, cyanocobalamin; OH-Cbl, hydroxocobalamin; AdoCbl, 5-deoxyadenosylcobalamin; Cbl, cobalamin; TMSOTf, trimethylsilyl triflate; DMBI, dimethylbenzimidazole; TCE, trichloroethene; *P. shermanii*, *Propionibacterium shermanii*; Ado(BzIm)Cbl, 5-deoxyadenosylbenzimidazolylcobamide; Ado(Im)Cbl, 5-deoxyadenosylimidazolylcobamide; RTPR, ribonucleoside triphosphate reductase; Tol, toluoyl; TMS, trimethylsilyl; TEA, triethylamine; DMF, dimethylformamide; Ac, acetyl; Bn, benzyl; Bz, benzoyl; Tr, trityl; TFA, trifluoroacetic acid; FMPT, 2-fluoro-1-methylpyridinium tosylate.

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1. Introduction

Various nucleoside/carbohydrate bearing species found in nature are important biomolecules. Nucleosides/carbohydrates play a vital role in biological systems and control a number of reactions in living cells. Naturally occurring ribonucleosides are almost always in the β -anomeric configuration with the exception of the vitamin B₁₂ family (Fig. 1), in which an α -ribonucleoside is included in the structure as the lower axial ligand.^{1,2} About a dozen enzymes, including the B₁₂ dependent ribonucleoside triphosphate reductase,³ ethanolamine ammonia lyase,⁴ glutamate mutase,⁵ and methylmalonyl CoA mutase require a B₁₂ cofactor. The cofactor for these reactions, 5-deoxyadenosylcobalamin (AdoCbl, co-enzyme B₁₂, Fig. 2), plays an important role by releasing a reactive species, 5-deoxyadenosyl radical, upon enzyme-induced cleavage of the carbon–cobalt bond. The mechanism of this enzymatic ‘activation’ of the co-enzyme, in which homolysis of C–Co bond catalyzed by some 10⁹ to 10¹⁴-fold remains unknown.

Vitamin B₁₂ derivatives are octahedral coordination complexes of Co(III) with a macrocyclic tetradentate equatorial ligand (the corrin ring), a lower axial 5,6-dimethylbenzimidazole (DMBI) ligand coordinated to cobalt through the imidazole (N-3) atom, and attached to a corrin ring side chain as an unusual α -ribonucleoside (to form the ‘nucleotide loop’),⁶ and various upper axial ligands (cyanide=vitamin B₁₂, CH₃=methylcobalamin, 5-deoxyadenosyl=AdoCbl). The Co–C linkage in AdoCbl was the first metal–carbon bond described in biological systems and is the key to the enzymatic activity of the B₁₂ co-enzyme. This report addresses recent advances in the synthesis of α -ribonucleosides, for which little prior literature exists, and which permits modification of the lower axial ligand of B₁₂ for mechanistic studies.

Vitamin B₁₂ derivatives with altered lower axial ligands are of interest as probes of the mechanism of action of co-enzyme B₁₂. In order to determine what role, if any, the lower axial ligand plays in the enzymatic activation of co-enzyme B₁₂, it is necessary to synthesize vitamin B₁₂ derivatives with altered axial nucleosides. Although such analogs may be obtained by guided biosynthesis,⁷ using fermentation of appropriate bacteria on media supplemented with the

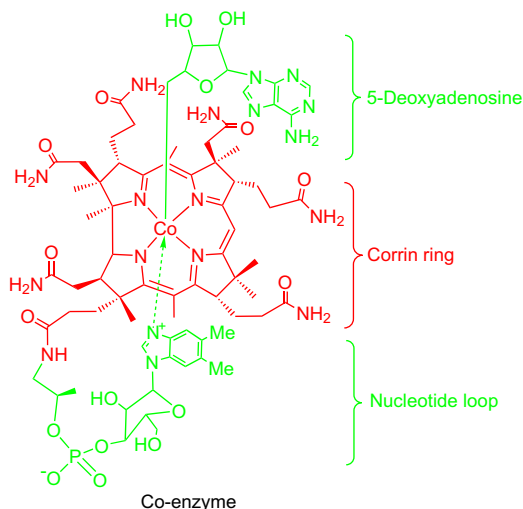


Figure 2.

desired axial base, analogs with indole axial nucleotides (which mimic the natural structure but lack the coordinating nitrogen) cannot be obtained, as these organisms fail to glycosylate indoles to make the required nucleoside precursor.⁸ Semisynthesis of such cobalamin derivatives is best achieved by coupling cobyrinic acid mixed anhydride with a nucleoside 3'-phosphodiester having an (*R*)-(-)-1-amino-2-propanol residue. For the synthesis of the latter nucleotide, the critical step is the synthesis of the nucleoside, which has the unusual α -*N*-glycosidic bond configuration.^{8–15}

5,6-Dimethyl- α -D-ribofuranosylbenzimidazole is one of the few naturally occurring α -ribonucleosides that has attracted significant attention due to its possible role in the activation of co-enzyme B₁₂.¹ A number of nucleoside and oligonucleotide analogs have been used in medicinal applications due to their diverse biological effects in human beings,¹⁶ but a majority of these compounds are analogs of β -nucleosides. β -Nucleosides are common in nature and can be prepared easily by established glycosylation methods. On the other hand, the use of α -nucleosides and their analogs is limited due to the limited availability of synthetic routes to these anomers. Thus, only a limited number of α -nucleosides are

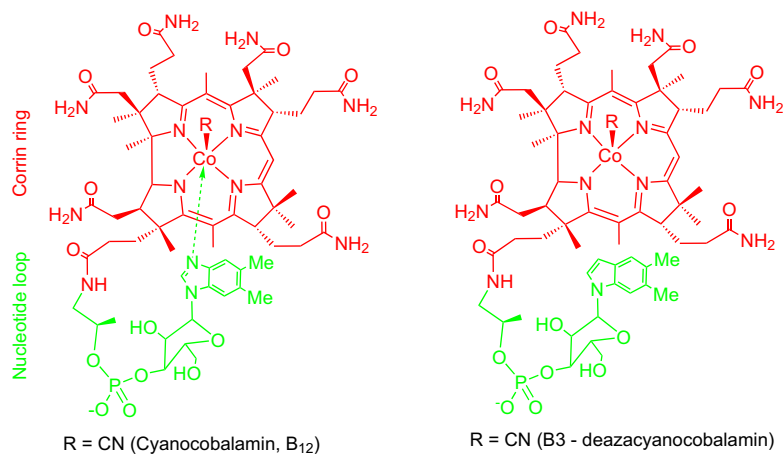


Figure 1.

known for their biological activity in human beings. α -2-Deoxythioguanosine has shown promising antitumor activity and been found to be less toxic compared to its β -counterparts.¹⁷ As α -ribonucleosides have not attracted much synthetic attention, most α -ribonucleosides arise as by-products of glycosylation reactions intended for the synthesis of β -ribonucleosides. Although some general glycosylation reactions produce up to 40–50% of the α -anomer, it is often difficult to separate the α -anomer from its β -counterpart by column chromatography or crystallization, due to their similar R_f values and chemical nature. Since indoles and indolines are not incorporated into cobalamin structure when the media of B_{12} overproducing bacteria are supplemented with them, it is necessary to prepare the α -nucleosides or nucleotides of indole, and their substituted derivatives for semisynthesis of B3-deaza derivatives of vitamin B_{12} . The replacement of the 5,6-dimethylbenzimidazole nucleotide¹⁸ with a 5,6-dimethylindole nucleotide loop will produce an exact replica of the cobalamin without a coordinating nitrogen and serve as a test for the role, if any, of axial ligand coordination in the enzymatic activation of AdoCbl.

Vitamin B_{12} has been widely used for targeting cancer cells and for incorporating various bioactive molecules.¹⁹ Many bioconjugates of vitamin B_{12} have been prepared and used as receptor modulating agents for treating cancer. Neoplastic disorders such as leukemia, sarcoma, myeloma, carcinoma, neuroma, melanoma, cancers of the lung, liver, breast, colon, cervix, and prostate can be treated by such receptor modulating agents.²⁰ B_{12} is a large water soluble biomolecule and structural alterations are possible without affecting the affinity to different transporter and receptor proteins. Recent uses of cobalamin and its conjugates as delivery vehicles for cytotoxic anticancer drugs and fluorescent tumor imaging agents for malignant cells have given hope for new therapeutics for the treatment of these deadly diseases. Vitamin B_{12} can be easily conjugated to anticancer and radiotracer drugs for transport to specific sites. Grissom and co-workers have attached a number of cytotoxic drugs to cobalamin as upper axial ligands or to the 5'-carbon of the ribose sugar (Fig. 3).^{19b–e} Such B_{12} -drug conjugates are nontoxic until they reach the target site. Other bioconjugates of B_{12} modified at the 5'-sugar carbon have been reported, and have affinity for intrinsic factor (IF) similar to that of CNCbl-e-COO⁻. The 5'-hydroxyl group has been activated

with 1,1-carbonyldiimidazole, 1,1-carbonyldi(1,2,4-triazole), or di(1-benzotriazolyl)carbonate and then added to aminoalkanes, diaminoalkanes, or alkanedihydrazides (Fig. 4) to produce CNCbl analogs.

Cobalamins and their derivatives have been used as efficient catalysts.²¹ The availability of the different oxidation states of cobalt in B_{12} results in the formation of Co-alkyl derivatives and also in the lability of the Co–C bond, which is easily cleaved thermally or photochemically. The reducing properties of the B_{12} have been widely explored in reductive dehalogenations.²² Polychlorinated ethylenes are among the most commonly found volatile organics in ambient ground water in the United States. B_{12} is involved in both the enzymatic and nonenzymatic dehalogenation reactions.²² Several microorganisms have been isolated recently that can convert perchloroethylene (PCE) into trichloroethene (TCE), and subsequently TCE into *cis*-1,2-dichloroethene. The enzyme responsible for this dechlorination reaction uses a vitamin B_{12} derivative as its active cofactor. While the mechanisms involved in reductive dehalogenation of TCE are still under investigation, it is clear that the availability of reduced oxidation states is crucial and the reduction pathway goes through *cis*-DCE formation.²³

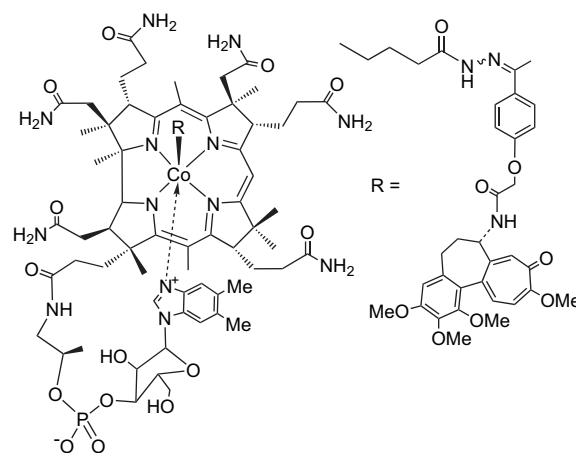


Figure 3. B_{12} colchicine analog.

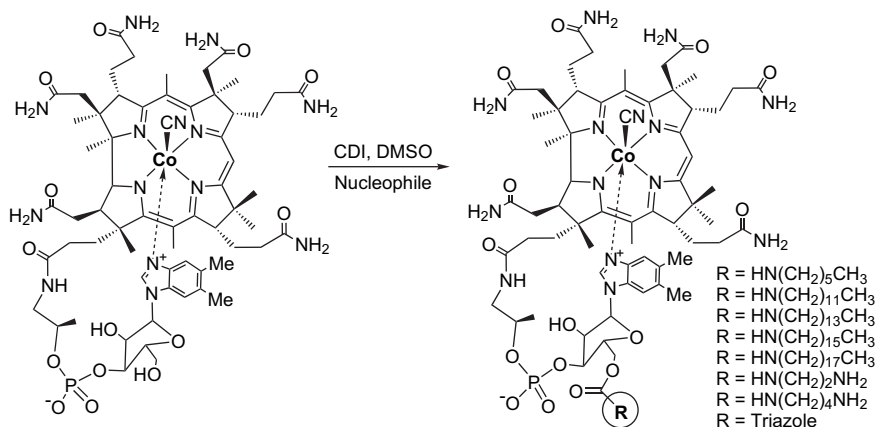


Figure 4.

2. Vitamin B₁₂ and α -ribonucleosides

Vitamin B₁₂ has attracted considerable attention since its discovery as the antipernicious anemic factor in liver.²⁴ 5,6-Dimethyl- α -D-ribofuranosylbenzimidazole of cobalamins is tethered to the corrin ring via the nucleotide loop, the unique features of which include the α -configuration of the *N*-glycoside and phosphorylation of the 3-hydroxyl group of the nucleoside sugar. The steric bulk of the lower axial ligand may be involved in triggering Co–C bond dissociation to form the adenosine radical, which is responsible for the enzymatic reactions involving 1,2-intramolecular substrate rearrangements or the reduction of ribonucleotides to deoxyribonucleotides. So far, only limited synthesis and characterization of rare α -ribonucleosides have been available in the literature. Our ongoing project for the semisynthesis/partial synthesis of cobalamin analogs²⁵ modified in the axial base (Fig. 5) requires the synthesis of the lower axial ligand of the nucleotide loop and has led to a variety of analogs of B₁₂^{1,2} in which the lower axial ligand is replaced with benzimidazole, imidazole, 4/5-fluoroimidazole, 4/5-bromoimidazole, and 4/5-methylimidazole.¹ These analogs have been obtained by fermentation of *Propionibacterium shermanii* on media supplemented with the desired bases ('guided biosynthesis').²⁶ These axially modified cobalamin derivatives are readily converted into co-enzyme B₁₂ analogs by reductive adenosylation with 5'-chloro-5'-deoxyadenosine. However, attempts to synthesize cobalamin derivatives with the indole or indoline axial nucleotides by guided biosynthesis have failed because the bacteria are incapable of incorporating these bases or their α -ribonucleosides into the nucleotide loop structure.²⁷

2.1. Biosynthesis of cobalamin derivatives

The cyano derivatives of the Cbl analogs, Co β -cyanobenzimidazolylcobamide (CN(Bzim)Cbl), Co β -cyanoimidazolylcobamide (CN(Im)Cbl), and Co β -cyano-4/5-fluoroimidazolylcobamide were readily obtained by 'guided biosynthesis' using fermentation of *P. shermanii* on media supplemented with desired base (Table 1), employing a modification of the method of Renz.²⁶ *P. shermanii* (ATCC 13673) was grown on solid microassay culture agar (DIFCO) in a test tube and made anaerobic by overlaying the culture with

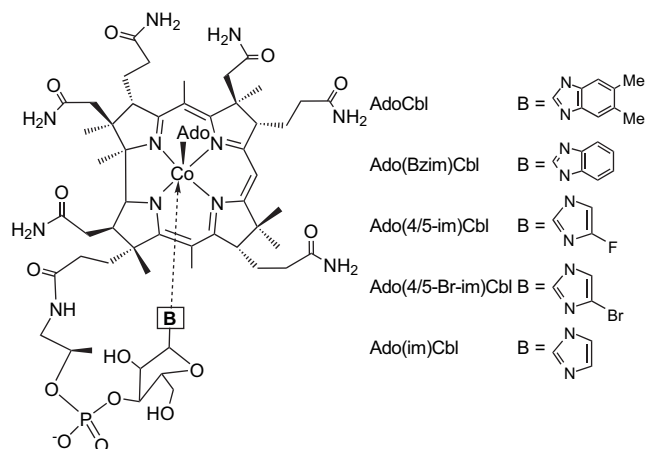


Figure 5. Analogs of co-enzyme B₁₂.

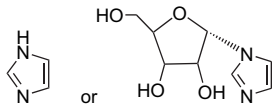
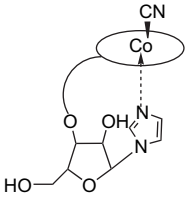

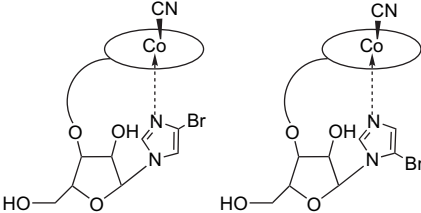

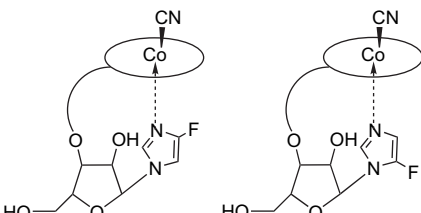
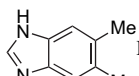
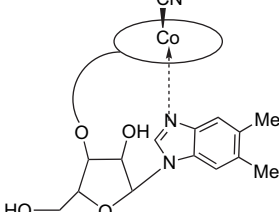

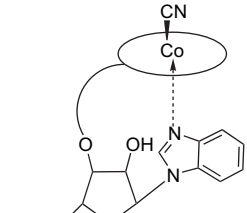
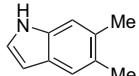
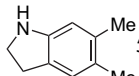


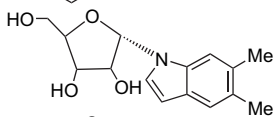
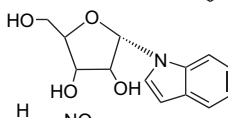

unsolidified, sterilized agar solution. After 5 days of growth at 30 °C, the culture was stored at 4 °C until use. The desired bases \approx 20–30 mg were added to the fermentation media as an ethanolic solution. After the usual workup the crude biosynthetic product was loaded onto a 4 \times 15 cm column of GC-161C absorbent and was washed with excess of water and 5% aqueous acetonitrile. The corrinoids were eluted with 15% aqueous acetonitrile, and the solvent was removed under reduced pressure. Crude products were further purified by semi-preparative HPLC using a 10 \times 250 mm C-8 column. To obtain high purity CN(B)Cbl, it was necessary to change the HPLC retention time of the product to effect separation from unidentified impurities. This was accomplished by conversion of the CN(B)Cbl to its aqua form by reduction with zinc in dilute acid followed by reoxidation in aerobic dilute HCl. Final purification by HPLC then permitted separation from the impurities that had coeluted with the cyano derivative. The purified aqua corrinoid was converted to its cyano derivative by reaction with KCN and desalting on a CG-161C column.

2.2. Role of axial ligand, 5,6-dimethylbenzimidazole α -ribonucleotide

The question of what role, if any, the 5,6-dimethylbenzimidazole ribonucleoside in B₁₂ plays in the activation of co-enzyme is a longstanding one. The effect of the steric bulk of the axial nucleoside base on the enzymatic activation of co-enzyme B₁₂ has been investigated using the co-enzyme analog in which imidazole replaces the bulky 5,6-dimethylbenzimidazole ligand (Ado(Im)Cbl, Fig. 1).¹ The effect of basicity of the axial nucleoside may be similarly probed using 5-substituted imidazole nucleosides. The roles of the D-ribose moiety and the bulky axial ligand of the nucleotide loop of adenosylcobalamin in co-enzymatic function have been investigated using two series of co-enzyme analogs bearing various artificial bases. The ribose moiety also plays important role by placing the dimethylbenzimidazole base in proper site. Cobalamin analogs in which the ribose is substituted by an aliphatic linker have been extensively studied by Toraya and co-workers.²⁸ Here, the phosphate and base were linked by di-, tri-, tetra-, and hexa-methylene spacer groups in the case of 5,6-dimethylbenzimidazole base and a trimethylene spacer group in the case of the imidazole base (Fig. 6). The authors found that these analogs show weaker coordination of the base to the cobalt atom, and were less active compared to AdoCbl (co-enzyme). There has long been interest in the question of whether the axial nucleotide plays a significant role in the activation of the co-enzyme, and this interest has only been heightened by recent findings that different AdoCbl-dependent enzymes manipulate the axial ligation of the co-enzyme in different ways.

Perhaps the most well studied of the proposed mechanisms for enzymatic activation of AdoCbl involving the axial nucleotide are the so-called 'mechanochemical triggering' mechanisms.²⁹ The classical version of mechanochemical triggering envisions ground-state destabilization of AdoCbl by enzymatic compression of the long (2.24 Å)³⁰ axial Co–N bond leading to a sterically induced increase in the upward fold of the corrin ring,³¹ and consequent steric pressure on the axial Co–C bond stretching and weakening it.

Table 1. Biosynthesis of analogs of AdoCbl using *Propionibacterium shermanii*²⁶

Base added to the medium	Analog	Ref.
		1
 4(5)-Bromoimidazole		1
 4(5)-Fluoroimidazole		1
 Dimethylbenzimidazole		1
 Benzimidazole		1
	Did not incorporate	27
 5,6-Dimethylindoline	Did not incorporate	27
	Did not incorporate	27
 Indoline	Did not incorporate	25
	Did not incorporate	25
	Did not incorporate	25
 4-NO ₂	Did not incorporate	25

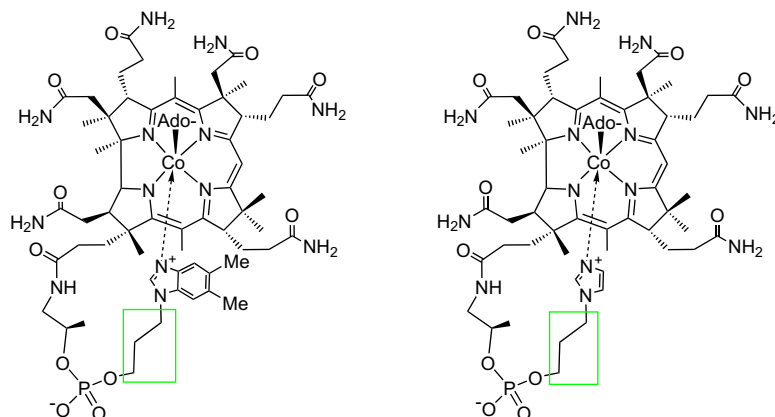


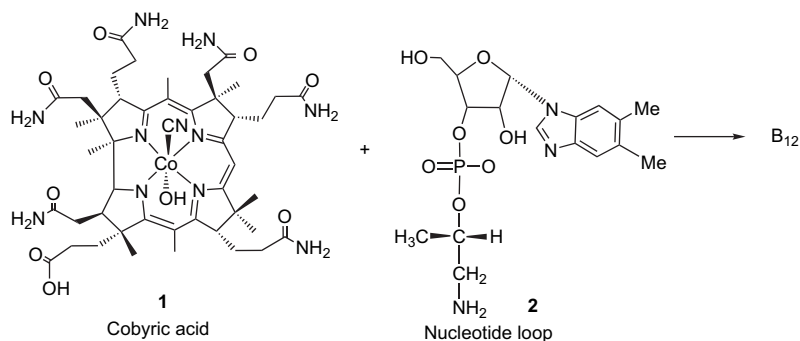
Figure 6.

2.3. Semisynthesis of vitamin B₁₂ and synthesis of 5,6-dimethylbenzimidazole α -ribonucleotide loop

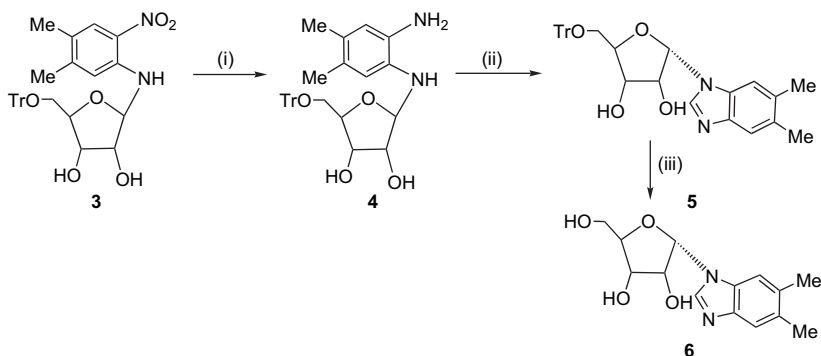
Woodward took on the enormous task of the total synthesis of vitamin B₁₂, a complex and sensitive biomolecule. The synthesis of the vitamin B₁₂ has been characterized as the most spectacular synthetic endeavor of the 20th century.³² Cobyric acid is the primary target of vitamin B₁₂ synthesis (Scheme 1), and can be used to prepare cobalamins derivatives modified at lower axial ligand. Acid hydrolysis of vitamin B₁₂ either by hydrochloric acid or TFA produces cobyric acid in moderate yields.³³ In order to synthesize the analogs of cobalamins modified at lower axial

ligand, this acid hydrolysis of vitamin B₁₂ remains the best option.

In 1952, Folkers and co-workers reported for the first time degradation products of B₁₂ by acid hydrolysis and they later compared the properties of the isolated product with synthetic α -ribazole (1-D- α -ribofuranosyl-5,6-dimethylbenzimidazole).³⁴ In order to prepare α -ribazole, 2-nitro-4,5-dimethyl-N-(5'-trityl-D-ribofuranosyl)aniline, **3**, was used as a starting material. Hydrogenation of the nitroriboside in methanol on a palladium catalyst yielded the amine, **4**, which was converted to the benzimidazole derivative, and subsequent removal of the trityl group afforded α -ribazole, **6** (Scheme 2).



Scheme 1.

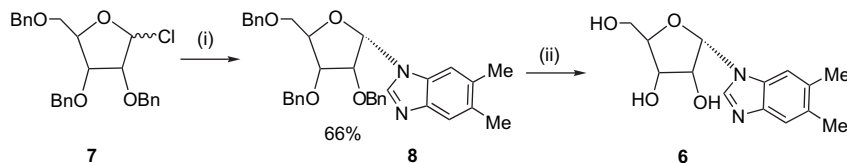


Scheme 2. Reagents and conditions: (i) methanol, palladium darco catalyst; (ii) isopropylformimino ether hydrochloride, refluxed, 2 h; (iii) ethanol/water, HCl, 1 h.

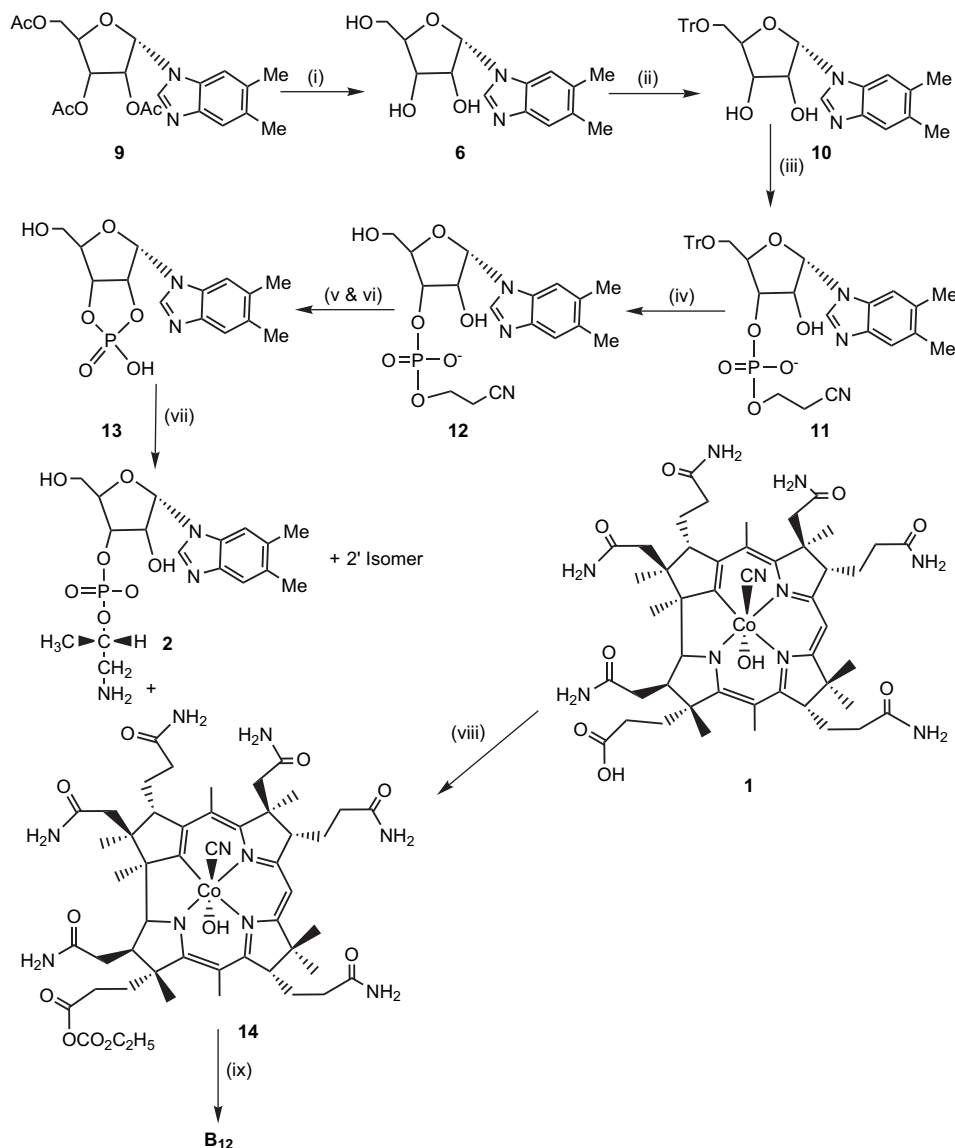
The use of 2,3,5-protected ribofuranosyl chloride for glycosylation of 5,6-dimethylbenzimidazole has been reported by Fletcher and co-workers.³⁵ Reaction of 2,3,5-tri-*O*-benzyl- α -D-ribofuranosyl chloride, **7**, with 5,6-dimethylbenzimidazole provides the desired nucleoside in good yields. Condensation of the halide in dioxane solution with slightly more than 2 mequiv of 5,6-dimethylbenzimidazole provides 1-(2,3,5-tri-*O*-benzyl- α -D-ribofuranosyl)-5,6-dimethylbenzimidazole **8** in 66% yield after column purification (Scheme 3). α -Ribazole **6** was easily obtained by catalytic debenzylation using 10% palladium on charcoal and palladium

chloride in excellent yields. Minor variations in the condensation of the sugar and base have been studied. Interestingly, the use of acetonitrile as a solvent decreases the yield of the desired α -nucleoside and the use of the silver salt of 5,6-dimethylbenzimidazole in acetonitrile decreases the yield to 25%.

The synthetic scheme for B₁₂ is shown in Scheme 4 starting from the protected 5,6-dimethylbenzimidazole ribonucleoside, **9**. The nucleotide loop was prepared in seven steps. Cobyrinic acid mixed anhydride was condensed with the



Scheme 3. Reagents and conditions: (i) dimethylbenzimidazole dioxane, 100 °C, 66%; (ii) methanol, palladium on charcoal, 94%.



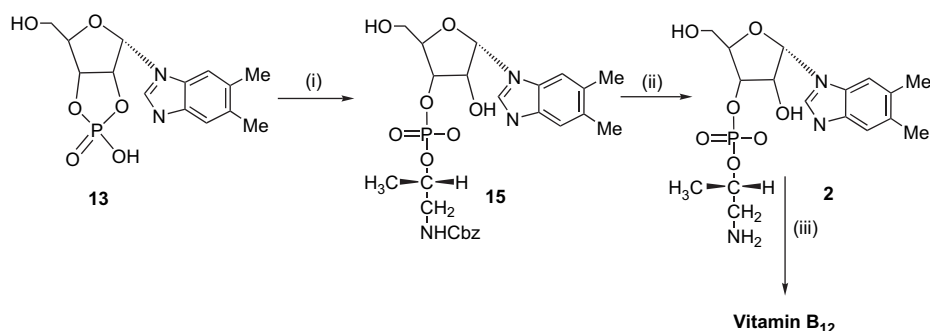
Scheme 4. Reagents and conditions: (i) NaOMe, methanol, rt; (ii) TrCl, pyridine, rt; (iii) β -cyanoethylphosphate, DCC, pyridine, rt; (iv) AcOH, 60 °C; (v) NH₄OH, 50–60 °C; (vi) DCC, formamide, 2 N, NH₄OH; (vii) potassium salt of D-1-amino-2-propanol; (viii) ethylchloroformate, DMF, TEA; (ix) TEA, DMF.

nucleotide loop in the presence of base. D-1-(Benzyloxycarbonylamino)propan-2-ol can also be condensed with cobyrinic acid in presence of acid (Scheme 5) and gives two isomeric phosphodiester, which can be easily separated by chromatography. The reaction of the mixture of 2'- and 3'-phosphodiester with cobyrinic acid afforded the corresponding 2'- and 3'-isomer of cobalamin. The 2'-isomer was found to be inactive and more labile compared to B₁₂ due to steric effects.^{33d}

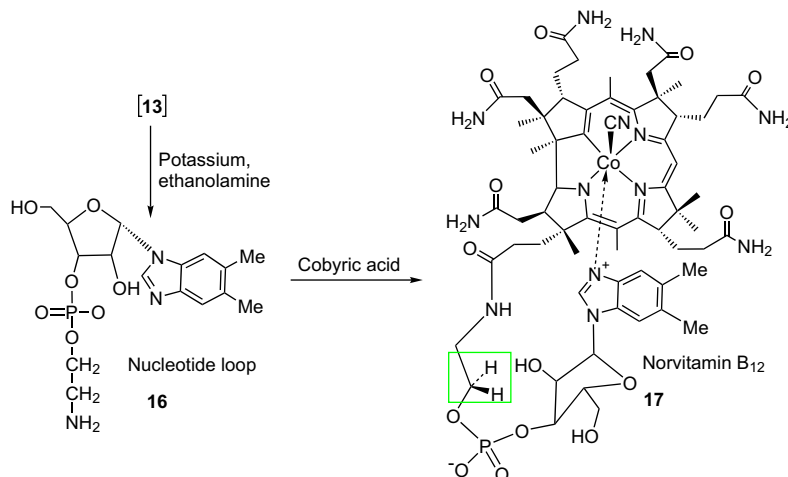
Norvitamin B₁₂, **17**, is the first known naturally occurring B₁₂ cofactor, which lacks the peripheral methyl group in the propanol residue of the nucleotide loop. Krautler and co-workers³⁶ prepared it by partial synthesis from cobyrinic acid and 2-aminoethyl-3-ribazoyl phosphate in 73% overall yields (Scheme 6). The nucleotide loop was synthesized by converting ethanolamine to its cyclic phosphate. The reaction produces the two isomeric 3'- and 2'-phosphate, in a 5:1 ratio and the isomers are easily separated by preparative TLC. The authors later postulated that the methyl group at periphery plays an important role by stabilizing the stable base-on conformation.

3. α -Ribonucleoside syntheses

While many routes exist for the synthesis of β -*N*-glycosides,³⁷ there are few methods available for the α -anomers.



Scheme 5. Reagents and conditions: (i) D-1-(benzyloxycarbonylamino)propan-2-ol; (ii) hydrogenolysis, Pd; (iii) cobyrinic acid mixed anhydride, base.



Scheme 6.

The synthesis of purine and pyrimidine nucleosides is generally carried out by silver salt,³⁸ chloromercury,³⁹ fusion,⁴⁰ phase transfer,⁴¹ sodium salt,⁴² or the boron trifluoride etherate⁴³ methods. These reagents are useful for constructing the β -glycosidic bond, but not the α -glycosidic bond, as they usually produce the β -nucleoside as the major product, and the α -nucleoside as a minor side product. The Vorbrüggen glycosylation⁴⁴ method is now frequently used for the preparation of the β -nucleosides (Scheme 7), using Friedel–Crafts catalysts to promote the glycosylation reaction. In this method, silylated purine, pyrimidine, or heterocyclic bases are treated with fully acylated sugars using trimethylsilyl triflate or tin(IV) chloride. Sugars protected with 2-acyloxy groups give *N*-glycosides with the β -configuration due to neighboring group participation.



Scheme 7. B=TMS-protected purine or pyrimidine bases.

Fletcher and co-workers³⁵ utilized partially benzylated sugars for making α -ribonucleosides. Condensation of

2',3',5'-tri-*O*-benzyl- β -D-ribofuranosyl chloride with 5,6-dimethylbenzimidazole produces up to 66% protected ribonucleoside and subsequent catalytic deprotection gives α -ribose in 94% yields. The authors also postulated that the presence of a participating acyl group at C-2 in glycosyl halides ends up with the formation of ribonucleosides in the β -configuration.

The reaction of 1-hydroxy sugars such as 2,3-*O*-(1-methylethylidene)-5-*O*-(triphenylmethyl)- α/β -D-ribofuranose⁴⁵ or 5-*O*-benzoyl-2,3-*O*-(1-methylethylidene)-D-ribofuranose⁴⁶ with trimethylsilylated benzimidazole and other nitrogenous bases, including nucleoside bases and azides, using 2-fluoro-1-methylpyridinium tosylate as condensing reagent (Scheme 8), provides predominantly α -ribonucleosides. However, as much as 47% of the β -anomer is obtained in some cases, requiring difficult separations of these isomeric mixtures by column chromatography and to date, no full characterization and isolation of these compounds have been reported. There are reports⁴⁷ of the use of ribofuranosyl chlorides for α -glycosylation, but these also produce mixtures of α - and β -*N*-glycoside. There had been not a single report of a reaction, which produces the α -nucleoside exclusively (Table 2) until our report¹² that the reaction of TMS-protected indoline bases (dimethyl indoline, 5-bromoindoline, and indoline) with 2,3-*O*-(1-methylethylidene)-5-*O*-(triphenylmethyl)- α/β -D-ribofuranose gives exclusively α -indoline ribonucleosides in excellent yield without the formation of any detectable β -ribonucleoside.

3.1. 2-Fluoro-1-methylpyridinium tosylate method

Mukaiyama and co-workers developed a stereoselective synthesis for α -ribonucleosides starting from 1-hydroxy sugars, using 2-fluoro-1-methylpyridinium tosylate.¹⁵ The sugar–pyridinium complex is believed to be more reactive toward nucleoside bases than to fluoride ion under reaction conditions (Scheme 8). The glycosylation reaction of dimethylbenzimidazole and 5-*O*-benzoyl-2,3-*O*-isopropylidene-D-ribofuranose was most stereospecific and afforded the α -anomer in a 90:10 ratio (Table 2). Protected theophylline generates maximum β -anomer due to the solubility of the

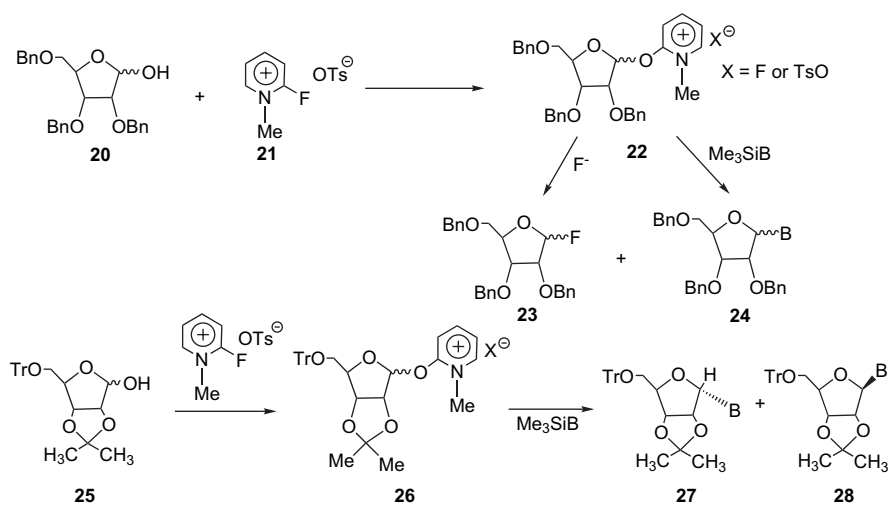
base anion under reaction conditions.¹⁵ The preferential reaction of the β -anomer, which remains in equilibrium with the α -anomer, with 2-fluoro-1-methylpyridinium tosylate fixes the configuration of the anomeric carbon. α -Nucleosides are considered to be the products of an S_N2 reaction with inversion at the anomeric center of the intermediate.

The extension of this methodology to other heterocyclic bases such as fluoro-, bromo- and iodoimidazole and indolines was investigated by Brown and co-workers and shown to result in complete regio- and stereoselectivity.^{9,12} The α -ribonucleosides were obtained in good yields without any complicated purification, and formation of 4-substituted haloimidazole ribonucleoside was not observed.

3.1.1. Synthesis of α/β -5-haloimidazole ribonucleosides.

In order to explore the role of basicity of the axial ligand in the activation of co-enzyme, we prepared a series of α -ribonucleosides of 5-haloimidazole in excellent yields.⁹ Reaction of TMS-protected 4(5)-fluoro-, bromo-, and iodoimidazole with 2,3-*O*-(1-isopropylidene)-5-*O*-(triphenylmethyl)- α/β -D-ribofuranose produced the 5-haloimidazole ribonucleosides predominantly in the α -configuration in 60–75% yield without any 4-substituted imidazole ribonucleoside (Scheme 9, Table 3). The 5-halo regioisomer was formed exclusively, while the α -anomer predominated by 3:1 to 5:1 ratio. The anomeric mixture was easily separated on flash silica gel column using an ether/benzene mixture. The crude reaction mixture showed only two products, the α - and β -anomer of 5-haloimidazole nucleosides, and no 4-halo substituted imidazole was detected.

There are reports on trimethylsilyltriflate-catalyzed glycosylation of 4(5)-substituted imidazole with protected ribose, which produces both 4- and 5-substituted β -imidazole ribonucleosides with the 4-regioisomers predominating (Scheme 10).⁶¹ The 4-isomer would be expected to be favored from steric considerations.⁶² However, in our synthetic protocols, the ¹H NMR of crude reaction mixtures showed only two products, the α -anomer (major) and β -anomer (minor) of the 5-haloimidazole ribonucleoside, as established using ¹H NOE after purification.



Scheme 8.

Table 2. Summary of the glycosylation reactions, which produce maximum α -ribonucleosides

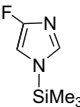
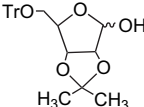
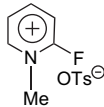
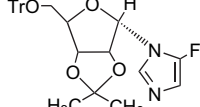
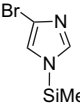
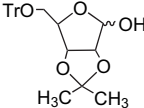
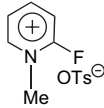
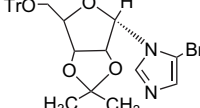
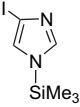
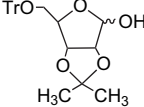
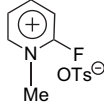
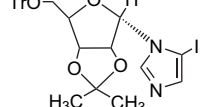
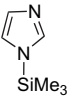
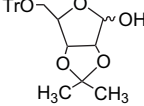
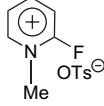
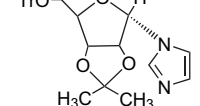
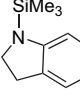
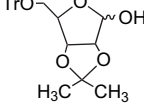
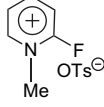
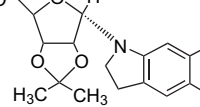
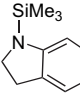
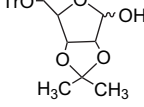
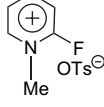
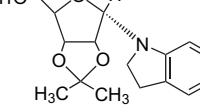
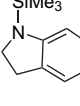
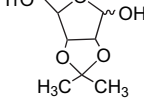
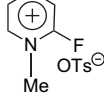
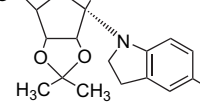
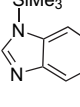
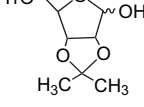
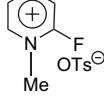
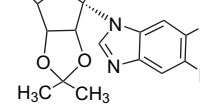
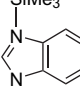
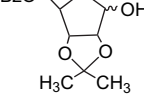
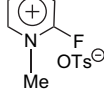
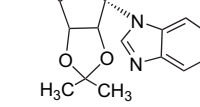
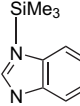
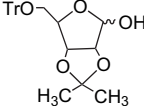
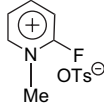
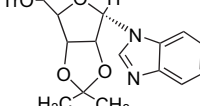
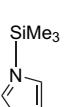
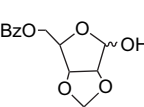
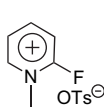
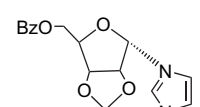
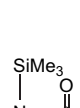
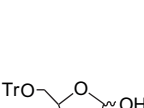

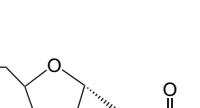
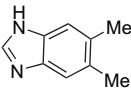
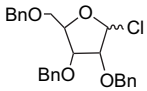
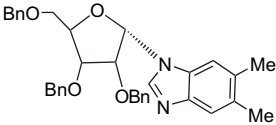
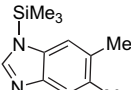
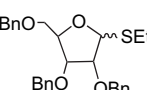
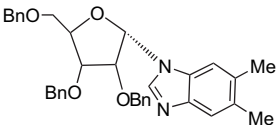
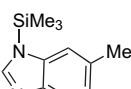
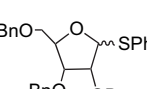
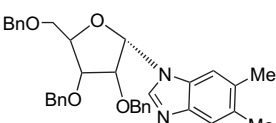
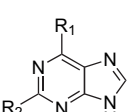
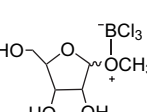
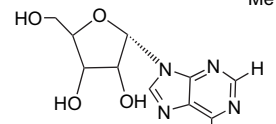
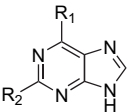
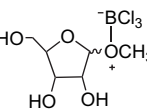
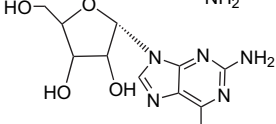
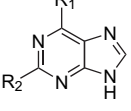
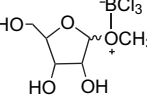
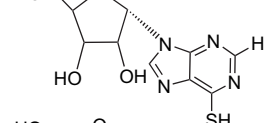
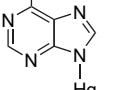
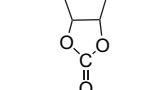
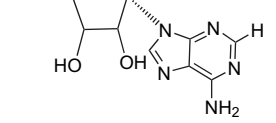
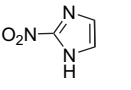
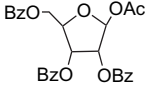
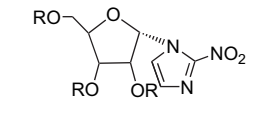
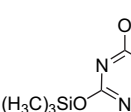
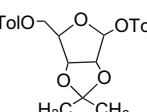
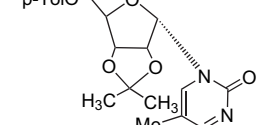
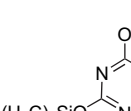
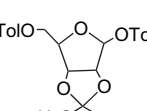
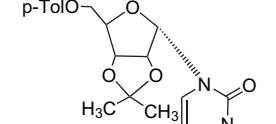
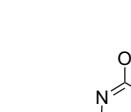
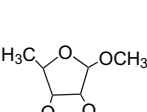
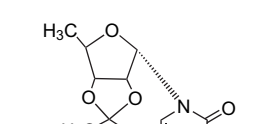
Base	Sugar	Catalyst	Nucleoside	Yields (%) (α)	Ref.
				75 ^a	9
				65 ^a	9
				65 ^a	9
				60 ^a	9
				96	12
				96	12
				92	12
				80 ^a	12
				89	15
				70 ^a	12
				86	15
				53	15

Table 2. (continued)

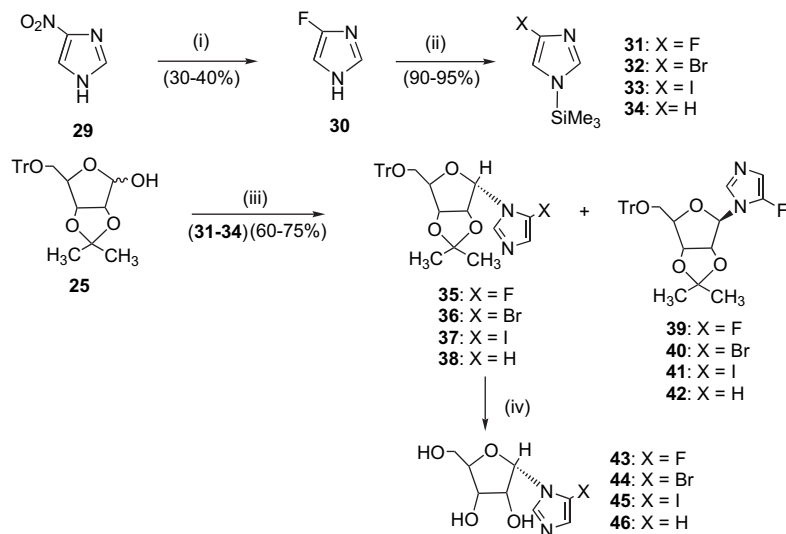
Base	Sugar	Catalyst	Nucleoside	Yields (%) (α)	Ref.
		None		66	35
		Molecular sieves 4 Å, NBS			47
		Molecular sieves 4 Å, NBS			47
		Pyridine		55	48
		Pyridine		55	48
		Pyridine		55	48
		—		24	49
		SnCl ₄ , Hg(CN) ₂		61	50
		TMSOTf		65	51
		TMSOTf		65	51
		TMSOTf		—	52

(continued)

Table 2. (continued)

Base	Sugar	Catalyst	Nucleoside	Yields (%) (α)	Ref.
		TMSOTf			53
		SnCl ₄			54
		SnCl ₄			55
		KI, dibenzo-18-crown-6			56
					57
		Cl(CH ₂) ₂ Cl			58
		Cs ₂ CO ₃ , NMP			59
		NaH, DMF			60

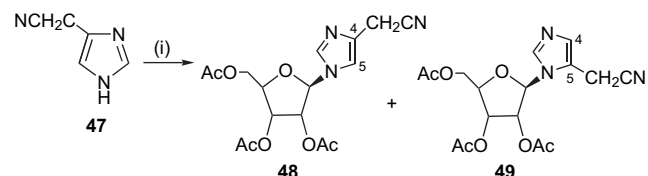
^a Isolated yields.



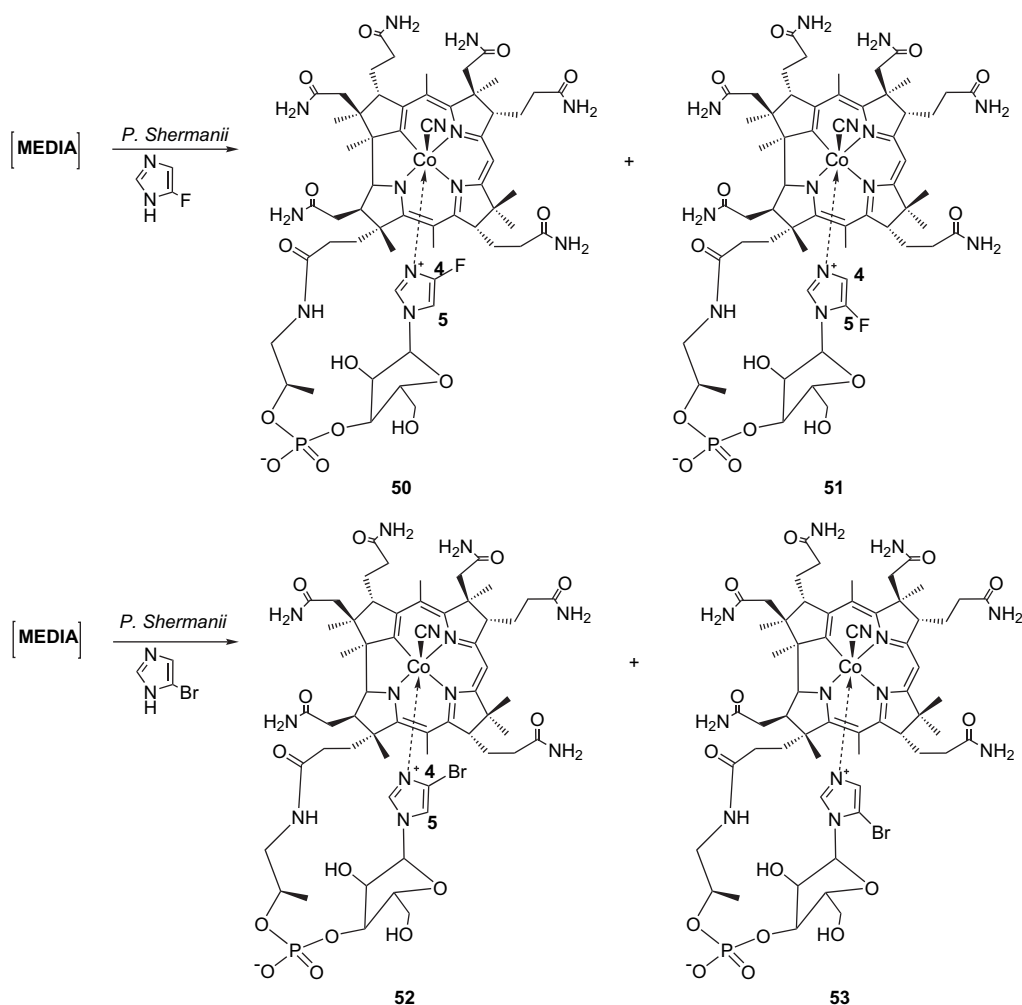
Scheme 9. Reagents and conditions: (i) NaNO₂, Zn dust, HBF₄, photolysis; (ii) 4-bromo-, 4-iodoimidazole, and imidazole, HMDS, reflux; (iii) 2-fluoromethyl pyridinium tosylate, DIEA, methylene chloride, -30 to 0 °C; (iv) TFA, rt, 90–95%, or methanol/HCl.

Table 3. Isolated yields and crude ratios of α/β -5-haloimidazole ribonucleosides

α -Ribonucleoside	Crude ratio α/β (%) ^a	Isolated yields (%)		Total yields (%)
		α	β	$\alpha+\beta$
5-Fluoroimidazole	80/20	75	15	90
5-Bromoimidazole	70/30	65	15	80
5-Iodoimidazole	80/20	65	20	85
Imidazole	70/30	60	18	78

^a Determined by NMR.**Scheme 10.** Reagents and conditions: (i) BSA, TMSOTf, 60 °C, protected ribose.

Interestingly, when 4(5)-fluoroimidazole was used to supplement the growth medium during the fermentation of *P. shermanii*, two analogs of cyanocobalamin were produced, one with 4-fluoroimidazole axial nucleotide and one with 5-fluoroimidazole axial nucleotide (Scheme 11).

**Scheme 11.**

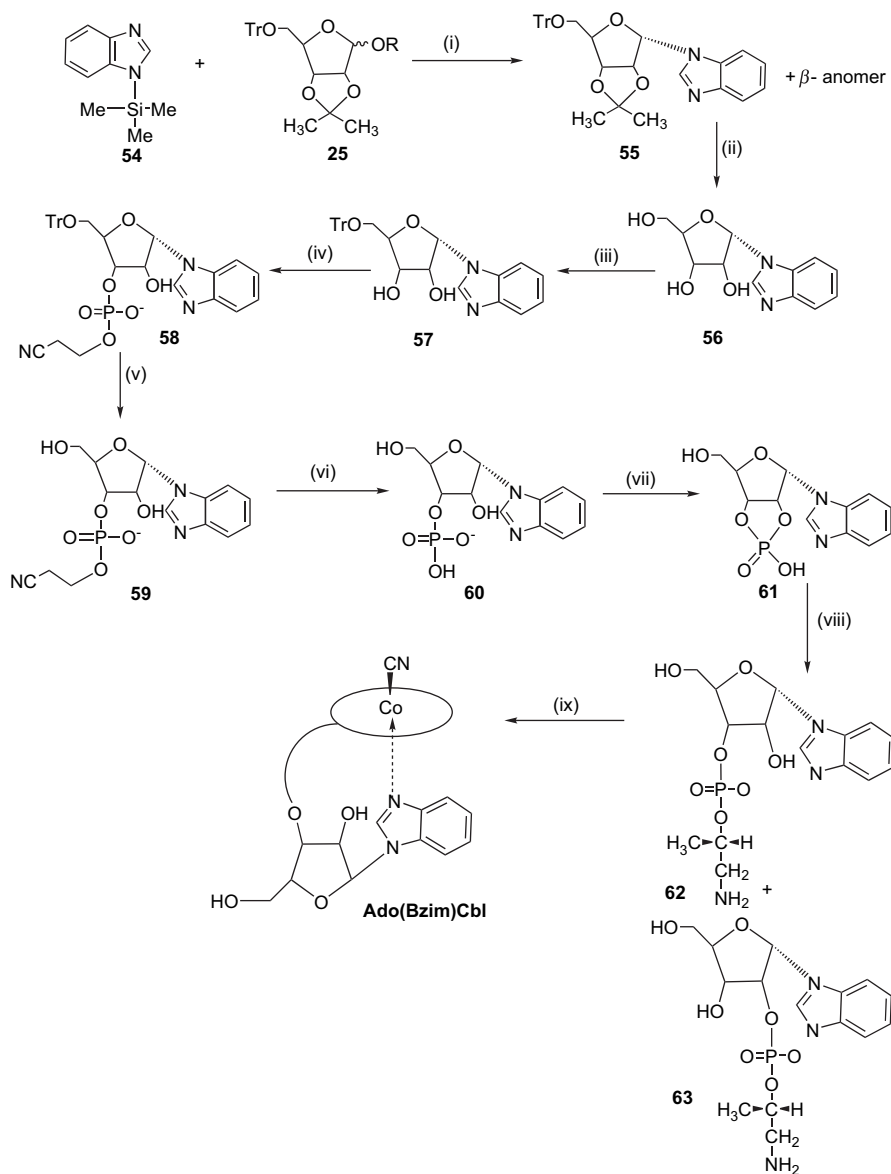
These two analogs were distinguished by the prominent ¹H NMR NOE cross-peak between the imidazole 5-H and the ribose anomeric proton in the 4-fluoroimidazole analog, which is absent in the ROESY spectrum of 5-fluoroimidazole analog. The structures of these isomers were confirmed by complete ¹H and ¹³C NMR assignments and mass spectroscopy. For further study, 4-fluoroimidazole α -ribonucleoside analogs can be made by the hydrolysis of the corresponding cyanocobalamin analogs.

For spectroscopic comparison the α - and β -ribonucleoside of imidazole was also prepared using the same methodology (Scheme 9). The absence of an NOE between the C(4)5 imidazole proton and the anomeric proton, readily observable in the imidazole nucleoside, confirms the structure of the 5-haloimidazole ribonucleosides. For the synthesis of the 5-fluoroimidazole α -ribonucleoside, the 4(5)-fluoroimidazole base^{63,64} was prepared from 4(5)-nitroimidazole in moderate yield by conversion to the diazonium salt followed by irradiation in aqueous tetrafluoroboric acid. After purification, the free base was converted to the TMS-fluoroimidazole by refluxing in HMDS for 10 h. For coupling of the TMS-protected base to the protected ribose, a mixture of 2-fluoro-1-methylpyridinium tosylate and 2,3-*O*-(1-isopropylidene)-5-*O*-(triphenylmethyl)- α/β -D-ribofuranose was stirred in methylene chloride at -30 to -10 °C in basic

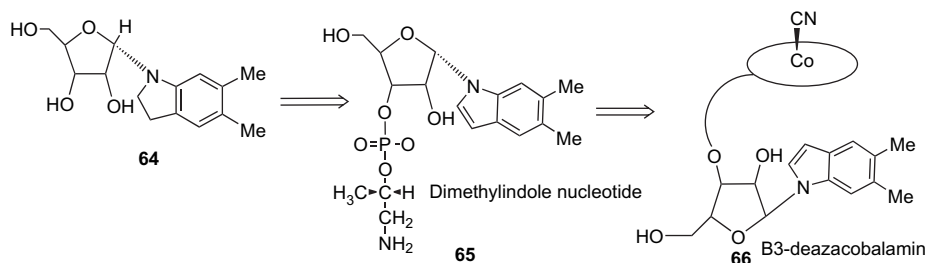
medium for 3 h and the TMS-protected base was added at 10 °C. The crude reaction mixture showed only the 5-halo-substituted α -ribonucleoside along with a minor amount of β -anomer by NMR. After flash chromatography, the crude mixture afforded 75% α -anomer and 15–20% β -anomer (Table 3). The other 5-haloimidazole ribonucleosides were obtained from the 4(5)-haloimidazoles similarly.

3.1.2. Semisynthesis of Co β -cyanobenzimidazolylcobamide. We successfully prepared Co β -cyanobenzimidazolylcobamide from α -benzimidazole ribonucleoside.⁶⁵ The α -ribonucleotide loop was prepared in eight steps starting from the benzimidazole base (Scheme 12). The desired α -benzimidazole ribonucleoside **55** was easily prepared by coupling 2,3-*O*-(1-methylethylidene)-5-*O*-(triphenylmethyl)- α/β -D-ribofuranose and the TMS-protected benzimidazole in presence of 2-fluoro-1-methylpyridinium *p*-toluene sulfonate. The partial synthesis of the cobalamin derivatives was

achieved by coupling the nucleotide with cobyrinic acid mixed anhydride obtained from vitamin B₁₂ hydrolysis.³³ The α -ribonucleoside was isolated in pure form as a crystalline solid in 70% yield by fractional crystallization using an ethyl acetate/hexane mixture. The protected nucleoside was deprotected without any difficulty using trifluoroacetic acid at ambient temperature or catalytic carbon tetrabromide in methanol in excellent yield. The deprotected ribonucleoside **56** was selectively protected at 5'-position with trityl chloride in pyridine at ambient temperature. Phosphorylation at the 3'-position was accomplished with β -cyanoethylphosphate and DCC in pyridine in 70–80% yield, followed by deprotection of the 5-hydroxyl using acetic acid at elevated temperature to give the corresponding alcohol, **59**. In the next step, the phosphate was deprotected using ammonium hydroxide at 50–60 °C, which afforded a mixture of the 2'- and 3'-nucleotide. Without any further purification, the nucleotide mixture was converted to the corresponding



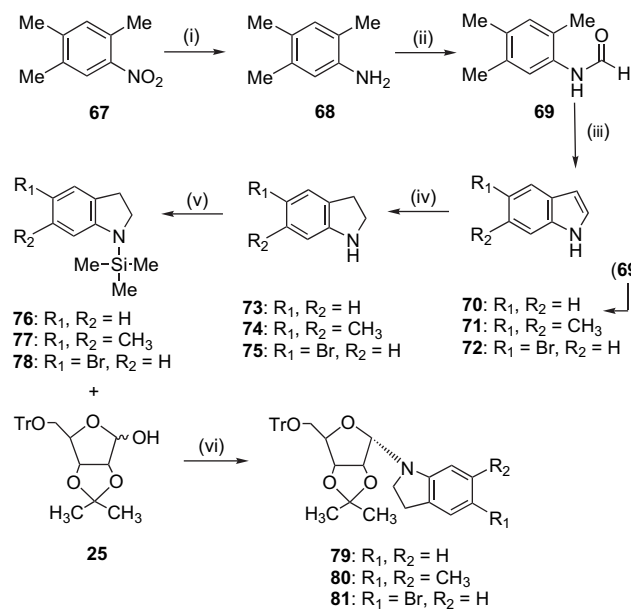
Scheme 12. Reagents and conditions: (i) 2-fluoromethyl pyridinium tosylate, DIEA, methylene chloride, -30 to 0 °C; (ii) TFA, rt; (iii) TrCl, pyridine; (iv) β -cyanoethylphosphate, DCC, pyridine, rt; (v) AcOH, 60 °C; (vi) NH_4OH , 50 – 60 °C; (vii) DCC, formamide, $2\text{ N NH}_4\text{OH}$; (viii) potassium salt of D-1-amino-2-propanol; (ix) cobyrinic acid mixed anhydride, TEA, DMF.



Scheme 13.

cyclic phosphate, **61**, which was isolated in good yield using DCC and ammonium hydroxide in a mixture of DMF and formamide. (*R*)-(-)-1-Amino-2-propanol was converted to its potassium salt for the coupling reaction, which produced two isomers, the 3-isomer (**62**) and the 2-isomer (**63**) in 70:30 ratio after reverse phase chromatography. Cobyrinic acid was converted to the mixed anhydride for final coupling with nucleotide loop. The semisynthesis/partial synthesis of B3-deazacobalamin (vitamin B₁₂ derivatives, Fig. 1) can also be achieved from 5,6-dimethylindoline α -ribonucleoside (Scheme 13). The α -indoline nucleoside was converted to the indole ribonucleoside using MnO₂ in presence of type 4 Å molecular sieves. Subsequent deprotection of the ribonucleoside and phosphorylation were easily completed by the procedures given in Scheme 13. Further synthesis of the nucleotide loop is in progress.

3.1.3. Indoline α -ribonucleosides. We reported¹² the first synthesis of the α -indoline ribonucleosides of 5,6-dimethylindoline, indoline, and 5-bromoindoline using 2-fluoro-1-methylpyridinium *p*-toluenesulfonate as a condensing reagent (Scheme 14). These glycosylation reactions produce the α -ribonucleosides exclusively without any β -nucleoside. Scheme 14 shows the synthetic route for the protected



Scheme 14. Reagents and conditions: (i) stannus chloride, HCl, reflux, 8 h; (ii) formic acid (80%), reflux; (iii) potassium metal, *tert*-butanol, reflux/thermal cyclization, 270–280 °C; (iv) sodium cyanoborohydride/AcOH; (v) butyllithium/trimethylsilyl chloride, –70 °C; (vi) 2-fluoromethylpyridinium tosylate, *N,N'*-diisopropylethylamine, methylene chloride, 0 °C.

indolines and their coupling to protected *D*-ribofuranose to form the α -indoline ribonucleosides. The dimethyl indole base, **71**, was synthesized in fairly good yield by a literature method⁶⁶ starting from the readily available 5-nitropseudoindole, **67**.⁶⁷ 5-Nitropseudoindole was reduced to 2,4,5-trimethylaniline, **68**, which was then converted to the formamide in 90% yield by refluxing in formic acid and the formamide was cyclized to the indole, **71**, in fairly good yield. The dimethylindole was converted to the dimethylindoline, **74**, in 90% yield using sodium cyanoborohydride in acetic acid at ambient temperature.⁶⁸ For the glycosylation reaction, the free base was protected with trimethylsilyl chloride.⁶⁹ 5,6-Dimethyl-1-trimethylsilyl-2,3-dihydro-1*H*-indole, **77**, was prepared in 98% yield from dimethylindoline at –70 °C. Similarly, the TMS-bromoindoline, **78**, was prepared in 95% yield from the corresponding 5-bromoindoline base.

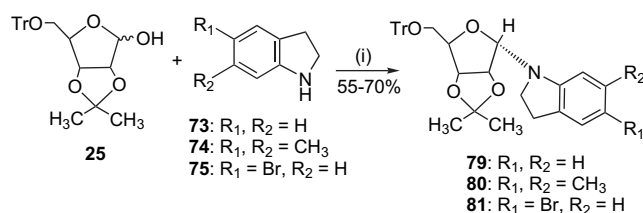
The silylated dimethylindoline bases were reacted with the anomeric mixture of the protected sugar 2,3-*O*-(1-methylethylidene)-5-*O*-(triphenylmethyl)- α/β -*D*-ribofuranose, **25** (a mixture of α - and β -anomer), using 2-fluoro-1-methylpyridinium *p*-toluenesulfonate as a condensing agent to produce the desired ribonucleosides in excellent yields. For this coupling reaction, 2-fluoro-1-methylpyridinium *p*-toluenesulfonate and the sugar were stirred in methylene chloride under basic condition, using *N,N*-diisopropylethylamine as a base, for 2–3 h at –30 °C and the silylated indoline was added to the reaction mixture at –10 °C in dry methylene chloride under an argon atmosphere.

These glycosylation reactions proceed smoothly and were easily monitored using NMR. These reactions were found to be highly regioselective and crude reaction mixtures showed exclusively α -ribonucleosides with no trace of β -nucleosides by NMR. After standard workup, the indoline α -ribonucleosides were easily purified by stirring in hexane at room temperature without any further column purification. The structure and anomeric configurations of these indoline α -ribonucleosides were determined by NMR and X-ray crystal diffraction studies.¹²

3.2. α -Indoline ribonucleosides by direct glycosylation

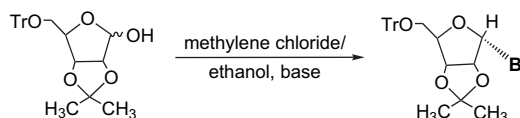
The indoline α -ribonucleosides, **79–81**, were also prepared¹¹ from unprotected indolines, **73–75**, by coupling to the protected ribose 2,3-*O*-(1-methylethylidene)-5-*O*-(triphenylmethyl)-*D*-ribofuranose, **25**, in dry ethanol/methylene chloride, in the presence of type 4 Å molecular sieves. These glycosylation reactions also produce exclusively the α -ribonucleosides in 55–70% yield without using any catalyst

(Scheme 15). The coupling reactions of the free bases and the protected sugar were carried out under a dry and inert atmosphere using either dry ethanol or methylene chloride. A dry solution of 2,3-*O*-(1-methylethylidene)-5-*O*-(triphenylmethyl)-D-ribofuranose in ethanol was added directly to dimethylindoline and 4 Å molecular sieves at room temperature and the mixture was heated for 5–6 h under an argon atmosphere while the reaction progress was monitored by TLC and NMR (Scheme 15). The reaction proceeded smoothly and after completion the mixture was cooled to room temperature, filtered, and thoroughly washed with ethanol. Using methylene chloride as a solvent, the observed yield was higher and there was no decomposition of the



Scheme 15. Reagents and conditions: (i) molecular sieves, ethanol/methylene chloride, 40–60 °C, 4–6 h.

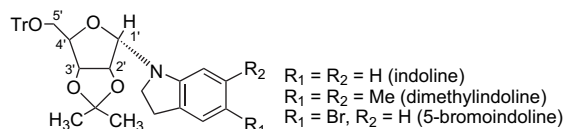
Table 4. Reaction conditions and yields of glycosylation of different bases with 2,3-*O*-(1-methylethylidene)-5-*O*-(triphenylmethyl)-D- α , β -ribofuranose



S. No.	Base (B)	Solvent	Rexn temp (°C)	Rexn time (h)	Yield ^a
1	Indoline	Ethanol	60–70	5–6	60
2	Dimethylindoline	Ethanol	60–70	4–5	65
3	5-Bromoindoline	Ethanol	60–70	6–7	55
4	Indoline	Methylene chloride	40	4	65
5	Dimethylindoline	Methylene chloride	40	4	70
6	5-Bromoindoline	Methylene chloride	40	5	60

^a Isolated yields.

Table 5. ¹H/¹³C NMR comparison of indoline ribonucleosides prepared by direct glycosylation^b/2-fluoro-1-methylpyridinium tosylate^a method (chemical shifts, ribose protons, δ in ppm)



S. No.	Compound (ribonucleosides)	Me ₁	Me ₂	5'	5''	4'	3'	2'	1'
1	Indoline ^a (¹ H NMR)	1.39	1.61	3.28	3.35	4.19	4.72	4.85	5.455
2	Indoline ^b	1.38	1.59	3.28	3.35	4.17	4.69	4.84	5.44
3	Indoline ^a (¹³ C NMR)	25.56	27.48	63.89	—	82.00	80.67	81.49	92.83
4	Indoline ^b	25.57	27.49	63.92	—	82.03	80.72	81.52	92.89
5	Dimethylindoline ^a (¹ H NMR)	1.38	1.60	3.26	3.32	4.19	4.64	4.80	5.41
6	Dimethylindoline ^b	1.39	1.62	3.29	3.33	4.21	4.67	4.83	5.43
7	Dimethylindoline ^a (¹³ C)	25.56	27.51	64.02	—	81.93	80.91	81.58	93.20
8	Dimethylindoline ^b	25.58	27.51	64.05	—	81.94	80.92	81.58	93.22
9	5-Bromoindoline ^a	1.38	1.60	3.24	3.41	4.14	4.64	4.77	5.32
10	5-Bromoindoline ^b	1.38	1.60	3.28	3.36	4.18	4.69	4.81	5.36

^a Reported ¹H NMR values in (δ in ppm).

^b Prepared by direct glycosylation.

sugar, while using ethanol as a solvent resulted in a sluggish reaction and decomposition of 2,3-*O*-(1-methylethylidene)-5-*O*-(triphenylmethyl)-D-ribofuranose at higher temperature. The reaction mixture showed only one isomer by NMR after workup. The reaction of dimethylindoline and 2,3-*O*-(1-methylethylidene)-5-*O*-(triphenylmethyl)-D-ribofuranose proceeds faster (Table 4) than the reaction with the other indoline bases. 5-Bromoindoline is less reactive and the longer heating time results in lower yields of the corresponding α -ribonucleoside.

The identity and α -configuration of these ribonucleosides were confirmed based on the corresponding α -indoline ribonucleosides, which were prepared by the 2-fluoro-1-methylpyridinium tosylate method and fully characterized by X-ray and 2D NMR spectroscopy.¹² The structure of these indoline ribonucleosides prepared by direct glycosylation was also confirmed by ¹H, ¹³C, and 2D NMR (COSY, HMQC, and NOESY). The signals for the methyl protons of the isopropylidene in **80** were present at δ 1.38 and 1.60 ppm and the anomeric proton signal was visible at δ 5.44 ppm (Table 5), the same as that reported for the indoline ribonucleosides⁸ prepared by the 2-fluoro-1-methylpyridinium tosylate method. The indoline methylene protons in the dimethylindoline ribonucleoside, **80**, showed a strong NOE with one of the isopropylidene methyls, which further supports the α -anomeric configuration. For further use as a precursor for B3-deazacobalamins (Fig. 1), these indoline ribonucleosides were converted to the corresponding indole ribonucleosides and all spectroscopic data were compared with the α -indole ribonucleosides, which were prepared using 2-fluoro-1-methylpyridinium *p*-toluenesulfonate method.¹⁴

3.3. Stannic chloride catalyzed glycosylation

An unusual product, 1-(2,3,5-tri-*O*-benzoyl- α -D-ribofuranosyl)-2-nitroimidazole (Scheme 16), was isolated during the synthesis of 1-(β -D-ribofuranosyl)-2-nitroimidazole, by Moffatt and co-workers.⁵⁰ Condensation of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose, **82**, with 2-nitroimidazole, **83**, in the presence of stannic chloride or mercuric cyanide produces 61% of the 2-nitroimidazole α -ribonucleoside, **85**.⁵⁰

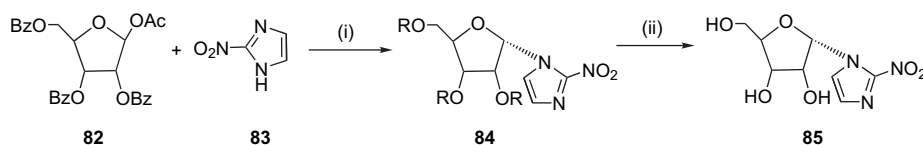
The predominant nucleoside in the reaction described should have the β -anomeric configuration due to participation by the 2'-*O*-benzoyl group, but the isolated product was, in fact, 1-(2,3,5-tri-*O*-benzoyl- α -D-ribofuranosyl)-2-nitroimidazole rather than the expected β -anomer.

3.4. Triflate method: synthesis of pyrimidine α -ribonucleoside

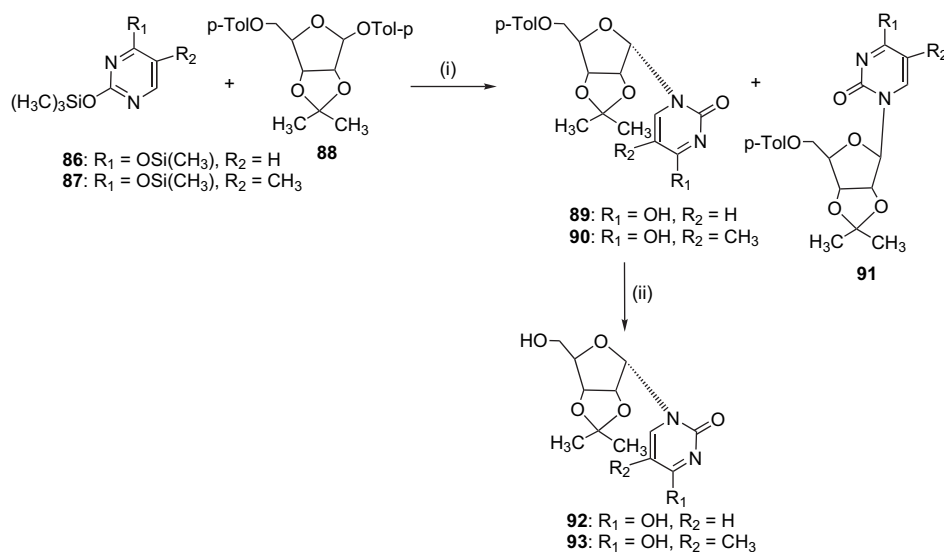
The synthesis of the pyrimidine α -ribonucleosides of thymine, uracil, and cytosine has also been reported.⁵¹ Condensation of TMS-protected pyrimidine bases, **86** and **87**, with 2,3-*O*-isopropylidene-1,5-di-*O*-*p*-toluoyl- β -D-ribofuranose, **88**, in 1,2-dichloroethane in the presence of trimethylsilyl triflate produces the desired ribonucleosides in fairly good yields (Scheme 17). This glycosylation method

produces mixtures of α/β -ribonucleosides of pyrimidines ranging from 9:1 to 6:1 ratio. However, only the protected thymine α -ribonucleoside, **90**, was isolated in pure form (in 65% yield) by crystallization from the α/β mixture. The α -ribonucleosides of cytosine and uracil were purified only after removal of toluoyl group. Only, 10–15% β -anomer was observed in crude mixtures by ¹H NMR. For purification purposes, the uracil and cytosine α -ribonucleosides were deprotected at 5'-position in order to separate the corresponding nucleosides from their β -counterparts and were isolated in 63 and 30%, respectively.

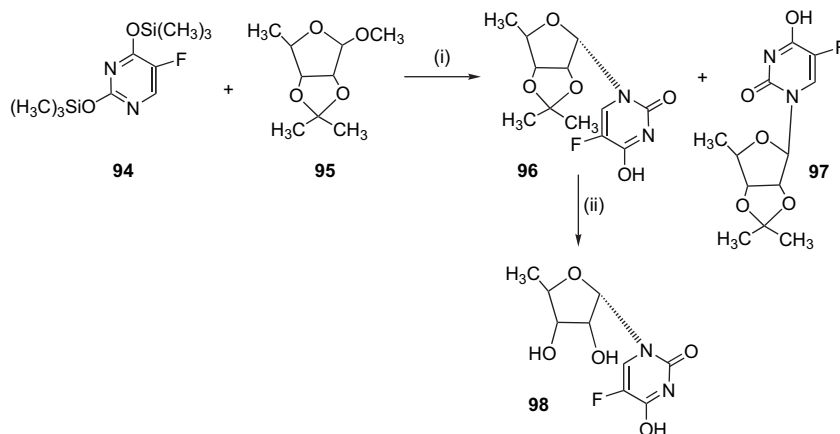
α -Fluorouridine derivative, **98** (Scheme 18), has been also prepared using trimethylsilyl triflate in good yield.⁵² Condensation of trimethylsilylated 5-fluorouracil with methyl 5-deoxy-2',3'-*O*-isopropylidene-D-ribose in the presence of



Scheme 16. Reagents and conditions: (i) stannic chloride/mercuric cyanide, 61%; (ii) hydrolysis.



Scheme 17. Reagents and conditions: (i) trimethylsilyl triflate, dichloroethane, reflux, 2 h; (ii) sodium methoxide, methanol, 30 min.

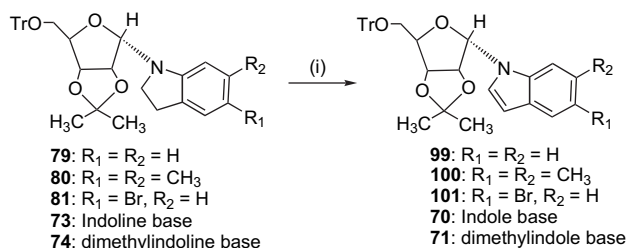


Scheme 18. Reagents and conditions: (i) trimethylsilyl triflate, methylene chloride, 15–20 h; (ii) TFA, rt.

trimethylsilyl triflate in methylene chloride at ambient temperature gave the α -ribonucleoside as a major product (Scheme 18). Catalytic removal of methoxy group at 1'-position produces the carbocation, which upon reaction with silylated fluorouracil in the absence of the neighboring group effect from the favored bottom side of the pentafuranoside leads to α -uracil nucleoside.

3.5. α -Indoline to α -indole ribonucleosides

A variety of α -indole nucleosides can be prepared from α -indoline nucleosides in excellent yield at moderate temperature using manganese dioxide and molecular sieves in benzene or methylene chloride.¹⁴ For the semisynthesis of cobalamins¹ (B3-deazacobalamin, Fig. 1), it is necessary to convert the indoline ribonucleoside to indole nucleoside for further manipulations under mild conditions. There are few reports on the dehydrogenation of the related β -indoline nucleosides to the corresponding β -indole nucleosides. Preobrazhenskaya⁷⁰ reported the dehydrogenation of 1-(2,3,5-*O*-tri-*O*-benzoyl- β -D-ribofuranosyl)indoline to the corresponding indole nucleoside using manganese dioxide in benzene at reflux for 15 h. However, the α -nucleosides are thermodynamically unstable and may isomerize to the corresponding β -nucleosides. Consequently, harsh reaction conditions for the dehydrogenation of the α -indoline nucleosides are problematic. The desired α -nucleosides do not survive in these drastic conditions and undergo polymerization. Dehydrogenation by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)⁷¹ does not give the desired nucleosides but gives charred products instead. In view of the above, we have developed a mild, high yield dehydrogenation method (Scheme 19) for these unstable α -indoline nucleosides using different solvents and molecular sieves. To compare the reactivity of these α -nucleosides, the indole and dimethylindoline bases were also dehydrogenated under similar condition and produced the indoles in similar yields (Scheme 19).



Scheme 19. Reagents and conditions: (i) MnO₂, molecular sieves, benzene, 40–50 °C/MnO₂, molecular sieves, methylene chloride, 30–40 °C.

1-(5-*O*-Triphenylmethyl-2,3-*O*-isopropylidene- α -D-ribofuranosyl)indoline, **79**, was dehydrogenated in either benzene or methylene chloride using MnO₂ in presence of type 4 Å molecular sieves. The reaction, which was monitored by TLC and NMR, proceeds rapidly and without decomposition or isomerization of the starting material. Dehydrogenation of dimethylindoline, **80**, and indoline nucleosides is complete within 1 h in benzene at 40–50 °C whereas the 5-bromoindoline, **81**, takes 1.5–3.0 h (Table 6). After completion, the reaction mixture was cooled to room temperature and filtered through Celite, and after the usual workup, the solvent was removed under reduced pressure. The NMR spectrum showed no organic impurities. The crude product was simply passed through a small silica gel column to remove the inorganic impurities. The reaction was repeated in methylene chloride at 30–40 °C using MnO₂ and molecular sieves and proceeded smoothly without any decomposition, in 95% yield (dimethylindoline). The reaction, however, proceeds much faster (1–1.5 h) in benzene than in methylene chloride. Without molecular sieves, the reaction was sluggish and required higher temperature and more time. The structures of the indole nucleosides were confirmed by ¹H NMR, ¹³C NMR, high-resolution mass spectroscopy, and 2D NMR (COSY and NOESY).

3.6. TMSOTf-mediated epimerization (β -thymidine to α -thymidine)

The reaction of protected thymidine with trimethylsilyl triflate affords α -thymidine **107**^{72a} which is a key intermediate for the synthesis of α -DNA^{72b-g} in 50% overall yields. The reaction of protected thymidine with trimethylsilyl triflate affords α -thymidine, **107** in 50% overall yields. Thymidine protected with triphenyl acetyl at 5' and a toluoyl group at 2' undergoes α/β -epimerization to give a 91:1 ratio of α to β -anomer (Scheme 20). The α/β ratio in epimerization reactions depends on the steric effect of the 5'-*O*-protecting group and the electronic effect of the 3' protecting group.

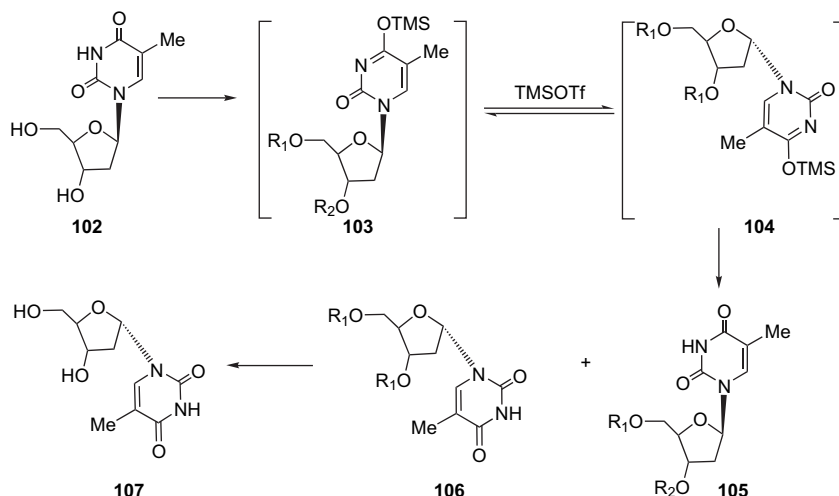
3.7. Boron trichloride complex method

Furukawa and co-workers used the BCl₃ complex of ribose **111** to prepare α -adenosine⁴⁸ **112** (Scheme 21). Octanoyl-adenine and the sugar were heated for 3 h in the presence of pyridine in chloroform. Increasing the amount of pyridine as an acid acceptor increases the yield of the product from 36 to 55%. Protected nucleosides were easily deprotected with sodium methoxide and subsequently purified on a Dowex column.

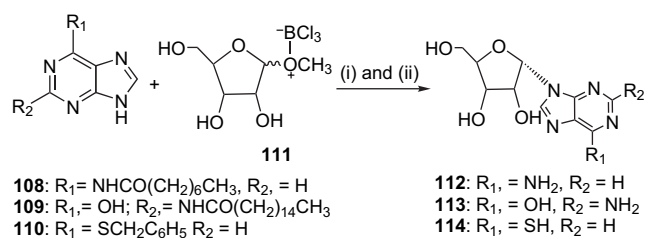
Table 6. Comparison of dehydrogenation of protected nucleoside by using benzene/methylene chloride in presence of MnO₂ and molecular sieves

S. No.	Reactant	Solvent	Rexn temp (°C)	Time (h)	Yield ^a (%)
1	Indoline nucleoside	Benzene	40–50	1–1.5	90
2	Dimethylindoline nucleoside	Benzene	40–50	1–1.5	94
3	Br-indoline nucleoside	Benzene	40–50	1.5–3.0	90
4	Indoline nucleoside	Methylene chloride	30–40	2–3	92
5	Dimethylindoline nucleoside	Methylene chloride	30–40	2–3	95
6	Br-indoline nucleoside	Methylene chloride	30–40	2–3	86
7	Indoline base	Benzene	40–50	1–2	90
8	Dimethylindoline base	Benzene	40–50	1–2	91

^a Isolated yields.



Scheme 20.



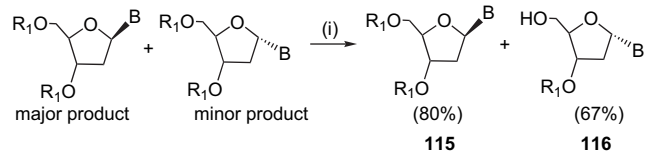
Scheme 21. Reagents and conditions: (i) pyridine, chloroform, reflux, 3 h; (ii) sodium methoxide.

3.8. Enzymatic separation of α/β -nucleosides

Quite recently, the enzyme lipase was used to separate mixtures of α - and β -deoxyribonucleoside through acylation or hydrolysis.⁷³ This method was successfully used to separate a mixture of protected α - and β -thymidine in good yields (Scheme 22). The authors used protected thymidine from industrial waste, which contains an 80:20 mixture of α - and β -protected thymidine. *Pseudomonas cepacia* (PSL-C) showed excellent regioselectivity for the hydrolysis of 5'-*p*-chlorobenzoate in the case of α -thymidine, while it was inactive with β -thymidine. After hydrolysis of thymidine, the mixture can be easily separated from β -protected thymidine.

4. Deprotection of α -ribonucleosides

Deprotecting these fragile α -ribonucleosides while retaining the α -configuration is crucial. Trityl (Tr), dimethoxytrityl (DMTr), and isopropylidene groups are commonly used for

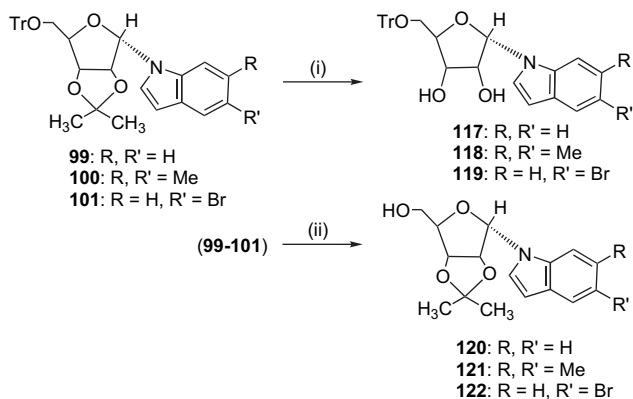


(Mixture of α and β thymidine 8:2 ratio)
 R₁ = *p*-Cl-benzoyl
 B = Thymine

Scheme 22. Reagents and conditions: (i) lipase, dioxane, 0.15 M phosphate buffer, 164 h, 60 °C.

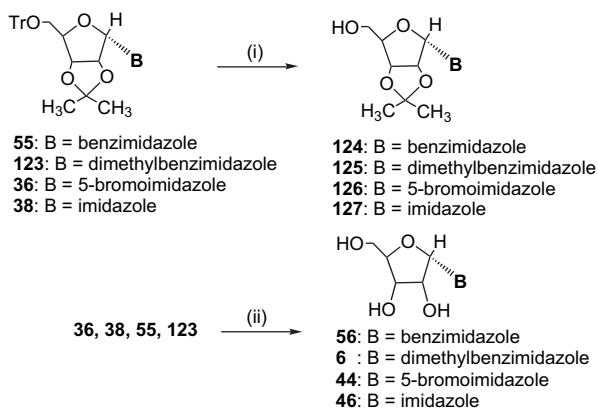
the protection of the 5-hydroxyl and 2,3-hydroxyl groups in both carbohydrate and nucleoside chemistries.⁷⁴ For α -ribonucleosides, protection of the 2', 3', and 5'-position is required for selectivity in the glycosylation reaction. These protecting groups are generally hydrolyzed under harsh conditions using strong protic and/or Lewis acids. Hydrochloric acid,⁷⁵ formic acid,⁷⁶ and trifluoroacetic acid (TFA)⁷⁷ are typically used for the deprotection of isopropylidene and trityl groups. Under these conditions, ribonucleosides with the α -anomeric configuration may undergo deglycosylation. Although α -ribonucleosides with dimethylbenzimidazole, benzimidazole, and imidazole bases survive these harsh reaction conditions, dimethylindole, indole, and other α -ribonucleosides when treated with neat TFA undergo deglycosylation, making it difficult to deprotect these ribonucleosides. In addition, strong protic acids such as TFA, HCl, and others are typically not selective under aqueous conditions and cause the cleavage of the other acid labile groups. Since insertion of an altered axial nucleoside into the cobalamin structure requires protection of the 5'-hydroxyl, chemoselective deprotection at the 2',3'-positions would be highly desirable. We have described the selective deprotection of trityl and isopropylidene groups using different acids in organic solvents.⁸

Upon treatment with formic acid/ether (2:3) at room temperature (Scheme 23) the indole ribonucleosides afforded the 2',3'-deprotected indole ribonucleosides (**117**–**119**) in fairly good yield, without affecting the trityl group. In contrast, the α -ribonucleosides of dimethylindole, indole, and 5-bromoindole, on treatment with aqueous acetic acid at 50–60 °C, afforded the corresponding 5'-deprotected α -ribonucleosides (**120**–**122**) without affecting the isopropylidene group. While it is known that (2',3'-*O*-isopropylidene- β -D-ribofuranosyl)-2,5,6-trichloroindole and other halogenated indole ribonucleosides produce 2',3'-deprotected indole ribonucleosides upon prolonged heating with acetic acid and water (4:1), for the α -indole ribonucleosides, we did not observe any 2',3'-deprotected products. Instead, prolonged heating of these α -indole ribonucleosides in acetic acid produced a charred product. Similarly, the isopropylidene group can be removed from the same substrates using 1% TFA in methylene chloride without affecting the trityl group.



Scheme 23. Reagents and conditions: (i) ether/formic acid (2:3), rt, 3–4 h; (ii) aqueous AcOH, 50–60 °C, 4–5 h.

1-(5-*O*-Triphenylmethyl-2,3-*O*-isopropylidene- α -D-ribofuranosyl)imidazole, -benzimidazole, -5-bromoimidazole, and -5,6-dimethylbenzimidazole ribonucleosides, however, react differently (Scheme 24). Here the trityl group can be easily removed by treating the protected ribonucleoside with formic acid/ether, heating with aqueous acetic acid,⁸ or using 1% TFA in methylene chloride without affecting the isopropylidene. However, these α -ribofuranosides were easily deprotected at the 5'- and 2',3'-positions without any deglycosylation using a mixture of TFA/H₂O (1:1) at ambient temperature.



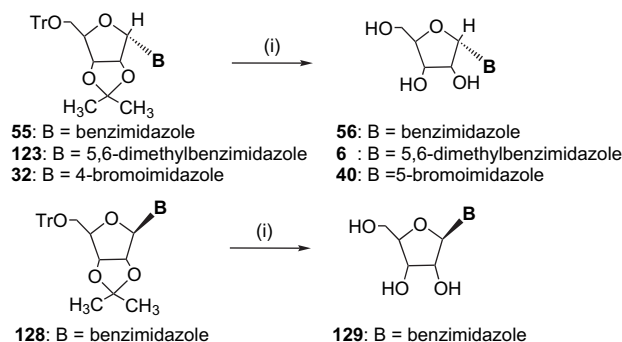
Scheme 24. Reagents and conditions: (i) ether/formic acid (2:3), rt, 3–4 h, or 1% TFA, methylene chloride, rt, 30 min or aqueous AcOH (80:20), 50–60 °C; (ii) 50% TFA/water.

4.1. Deprotection of α -imidazole/benzimidazole ribonucleosides by catalytic carbon tetrabromide initiated photolysis

While trityl and dimethoxytrityl ethers are selectively deprotected to the corresponding alcohols by CBr₄ in methanol at reflux under neutral reaction conditions,⁷⁷ at these temperatures, imidazole ribonucleosides isomerize from the α -anomer to the β -anomer. We have developed a mild and highly efficient deprotection of trityl and isopropylidene protecting groups without any deglycosylation or anomerization, in high yield, using sub-stoichiometric amounts of carbon tetrabromide. α -Ribonucleosides of imidazole and benzimidazole can be readily deprotected by photoirradiation using catalytic carbon tetrabromide (CBr₄) in methanol

at ambient temperature. Although selective deprotection of trityl versus isopropylidene groups of β -ribofuranosides and sugars has been achieved using photolysis,⁸¹ our photolysis protocol shows no selectivity in trityl/isopropylidene deprotection for the α -ribofuranosides.

The photolysis reactions were carried out in glass vials using a high pressure Xe–Hg photolysis lamp (254 nm principal wavelength) for 30 min⁻¹ 0 h at ambient temperature (Scheme 25). Complete conversion of the starting material was confirmed by TLC (methanol/methylene chloride 8:2) as well as by ¹H NMR. Increasing the amount of carbon tetrabromide from 0.05 to 0.5 equiv not only decreased the reaction time but also decreased the observed yield (Table 7). β -Ribonucleosides of benzimidazole, dimethylbenzimidazole, and 2,3-*O*-(1-methylethylidene)-5-*O*-(triphenylmethyl)- α/β -D-ribofuranose were also deprotected under similar conditions in yields of 80, 85, and 80%, respectively.



Scheme 25. Reagents and conditions: (i) methanol, carbon tetrabromide, 20 °C, photolysis.

In contrast, deprotection of the α -ribofuranosides of imidazole and 5-bromoimidazole with excess carbon tetrabromide (1–3 equiv) at reflux in methanol produced mixtures of α - and β -anomer (Table 8). These reactions take 5–6 h for complete removal of trityl and isopropylidene groups. With less carbon tetrabromide the reaction is very sluggish and complete deprotection takes 10–12 h. However, in the case of the benzimidazole and 5,6-dimethylbenzimidazole α -ribofuranosides, we observed only 5% anomerization under reflux

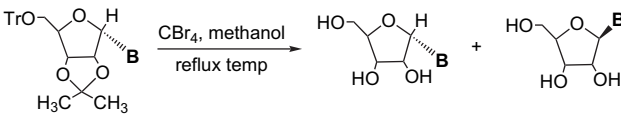
Table 7. Photolysis reaction conditions and yields

S. No.	Reactant (B)	CBr ₄ ^a	Reaction time	Yield ^b (%)
1	α -Benzimidazole	0.05	1.0 h	95
2	α -Benzimidazole ¹⁹	0.5	20 min	75–80
3	α -5,6-DMBz ^c	0.05	1.0 h	98
4	α -5,6-DMBz ^c	0.5	10–20 min	75
5	α -Imidazole	0.05	50 min	86
6	α -5-Bromoimidazole	0.05	1.0 h	90
7	β -Benzimidazole	0.05	1.0 h	85
8	β -5,6-DMBz ^c	0.05	1.0 h	85

^a Equivalent of CBr₄.

^b Isolated yields.

^c 5,6-DMBz = 5,6-dimethylbenzimidazole.

Table 8. Reaction conditions and yields under reflux conditions in methanol and carbon tetrabromide


S. No.	Reactant (B)	CBr ₄ ^a	Reaction time (h)	Yield ^b of α-anomer (%)	Yield ^b of β-anomer (%)
1	α-Benzimidazole	1–2	5–6	95	0
2	α-Benzimidazole	0.5	10–12	85	5
3	α-5,6-DMBz ^c	2	5–6	75	0
4	α-5,6-DMBz ^c	0.5	10–12	70–80	5
5	α-Imidazole	2	4–5	60–70	30
6	α-5-Bromoimidazole	3	4–5	80	20

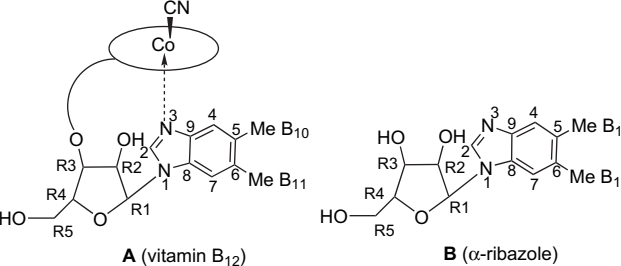
^a Equivalent of CBr₄.^b NMR yields.

conditions, possibly due to the more bulky bases compared to the imidazole and 5-bromoimidazole α-ribonucleosides.

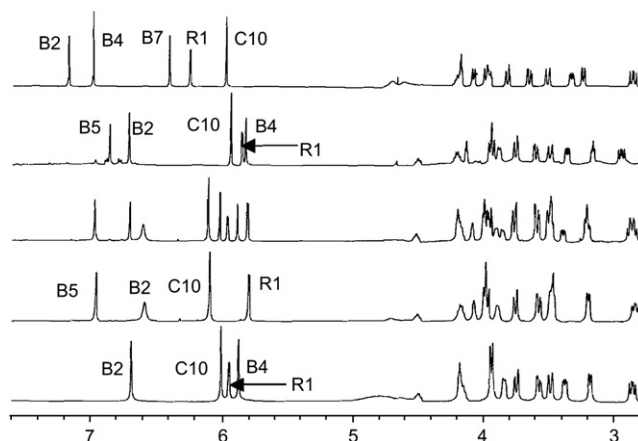
5. NMR and X-ray structures of α-ribonucleosides

5.1. Structure of α-ribazole

The anomeric configuration of nucleosides is generally determined by ¹H and ¹³C NMR spectroscopies. Due to the inclusion of α-ribazole in B₁₂, several NMR studies have appeared.⁷⁸ The structure of vitamin B₁₂ and its analogs has been extensively studied by NMR and X-ray crystallography. The α-ribonucleoside in B₁₂ shows notable changes in the NMR chemical shift at the ribose 1'-position and the base 2'-position (imidazole) due to steric effects and the anisotropic effect of cobalt (Table 9). The anomeric proton of ribazole coordinated to cobalt in B₁₂ is shifted slightly downfield compared to free ribazole, and a similar trend is seen for the anomeric carbon. Regarding the second factor, extensive NMR and structural studies of cobaloxime model

Table 9. ¹H and ¹³C chemical shifts for included ribazole and free ribazole


Atom	A (Vitamin B ₁₂)		B (α-Ribazole)		
	δ ¹ H	δ ¹³ C	δ ¹ H	Δ Hz	Δ ¹³ C
B2	7.10				
B4	6.51	145.67	7.50		121.29
B7	7.28	119.3	7.37		135.23
B10	2.27	114.3	2.35		22.20
B11	2.26	22.20	2.34		21.91
R1	6.36	21.91	6.33	4.00	88.89
R2	4.28	89.8	4.55	4.40	74.17
R3	4.73	71.7	4.43	7.20	73.07
R4	4.40	75.9	4.36	4.30	85.85
R5	3.93/3.75	84.9	3.77/3.92		63.67
		63.3			

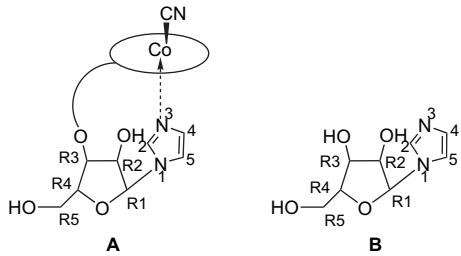
**Figure 7.** From top lower field region ¹H NMR of B₁₂, CN(Im)Cbl, mix of CN(4/5-Br-Im)Cbl, CN(4-Br-Im)Cbl, and CN(5-Br-Im)Cbl.

complexes by Marzilli and co-workers⁷⁹ have found that the cobalt atom magnetic anisotropy has a significant effect on the chemical shifts of nearby atoms in base-on cobalamins. Brown and Hakimi studied the magnetic anisotropy of a series of base-on cobalamins and observed a significant trend in ¹³C NMR chemical shifts of the carbon atoms of the nucleotide loop.⁸⁰ These studies also suggest that changes in the upper axial ligand will have a much larger effect on the magnetic anisotropy of the cobalt atom than changes in the lower axial Co–N bond distance and that distortions of the equatorial ligand (due to bulky upper axial ligand) also significantly affect cobalt magnetic anisotropy. In B₁₂, the ribose 3'-proton and carbon chemical shifts of the α-ribazole moiety are altered in the ¹H and ¹³C NMR, because the ribose C3 is directly attached to phosphodiester linkage. In vitamin B₁₂, the R-3 (ribose 3') proton is 0.4 ppm deshielded compared to the free nucleoside (Fig. 7), and the carbon is also shifted 1.0 ppm downfield compared to free α-ribazole (Table 9). The CN(Im)Cbl R-3 carbon is shifted 2.6 ppm downfield compared to the free nucleoside^{7a} (Table 10).

5.2. Imidazole and 5-haloimidazole α-ribonucleosides

The structures of the imidazole and 5-haloimidazole α-ribonucleosides were established by NMR and X-ray crystallography. The methyl proton chemical shifts and chemical shift separation for the *endo*- and *exo*-methyl groups of the isopropylidene in protected nucleosides have been extensively used to determine the anomeric configuration of nucleosides.^{81,82} Imbach⁸³ suggests that the difference in the ¹H NMR chemical shifts between the two methyl signals of the isopropylidene group, Δδ, can be used to distinguish between the α- and β-anomers such that 0.18 < Δδ < 0.23 for the β-anomers and 0 < Δδ < 0.10 for the α-anomers (Fig. 9, α-imidazole nucleoside). Coupling constant trends for H1'–H2' are also used to determine the configuration of α/β-ribonucleosides. Generally, the coupling constant for an α-ribonucleoside is higher (Table 11) than that of a β-ribonucleosides (β, J_{1'-2'} = 1–3.5 Hz, α, J_{1'-2'} = 3.5–8.0 Hz).

From the X-ray crystal structure of 5-fluoroimidazole ribonucleoside (Fig. 8), the anomeric proton and the fluorine atom are separated by 2.386 Å in space. A similar distance

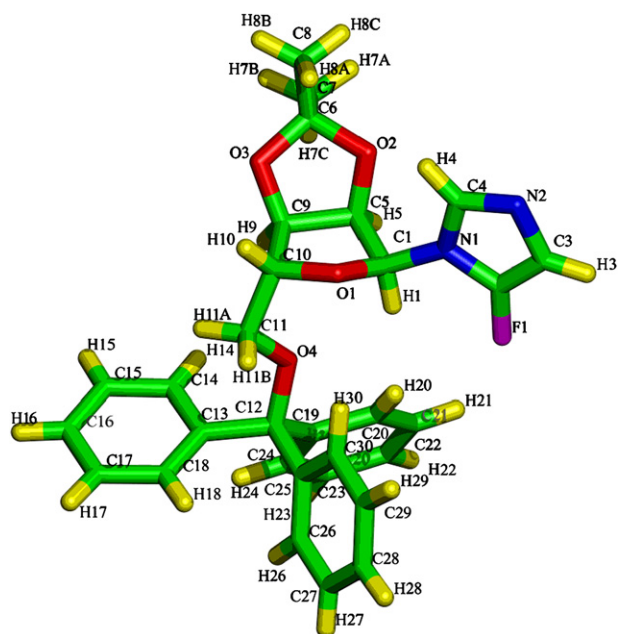
Table 10. ^1H and ^{13}C NMR chemical shifts for imidazole cobalamin CN(Im)Cbl included ribonucleoside and free α -imidazole ribonucleoside


Atom	A			B		
	δ ^1H	Δ Hz	Δ ^{13}C	δ ^1H	Δ Hz	Δ ^{13}C
B2	6.82		139.2	8.89		136.58
B4	5.96		128.6	7.63		123.58
B5	6.97		122.2	7.49		121.75
B10	—		—	—		—
B11	—		—	—		—
R1	5.97	t, 4.0	91.5	6.24	4.5	91.55
R2	4.24	t, 3.5, 4.5	72.2	4.64	5.3	88.81
R3	4.61	dt, 8.4, 8.4,4.5	75.5	4.48	—	72.90
R4	3.99		84.7	4.34	—	74.21
R5	3.69, 3.86	dd, 2.5, 12.5	62.8	3.79	8.0, 10	63.73

Table 11. $^1\text{H}/^{13}\text{C}$ NMR chemical shifts of ribose sugar for α/β -5-haloimidazole ribonucleosides

S. No.	Compound	1'	$J_{1'-2'}$ Hz	2'	3'	4'
1	α -5-Fluoroimidazole	6.21/85.9	4.0	4.96/80.2	4.70/82.5	4.42/82.2
2	α -5-Bromoimidazole	6.49/88.5	4.0	5.0/79.9	4.70/82.0	4.42/82.06
3	α -5-Iodoimidazole	6.46/90.5	4.5	5.0/79.8	4.72/82.6	4.42/81.9
4	α -Imidazole	6.20/88.5	4.03	4.92/80.5	4.77/82.4	4.39/82.1
5	β -5-Bromoimidazole	5.87/91.2	2.8	4.86/85.1	4.74/80.8	4.37/84.6
6	β -Imidazole	5.76/91.92	—	4.79/81.27	4.74/85.31	4.38/84.45

is expected between the anomeric proton and the C-5 proton of imidazole in its α -ribonucleoside, so there is a strong NOE between those protons (Fig. 10 and 11). In the ^1H NMR, a doublet was observed at 6.54 ppm for the imidazole H-4

**Figure 8.** X-ray structure of α -5-fluoroimidazole nucleoside **35**.

($^3J_{\text{HF}}=6.9$ Hz). In the ^{19}F NMR, a doublet was observed at -154.6 ppm with J value of 7.5 Hz, while 4(5)-fluoroimidazole itself has a ^{19}F NMR doublet at -136.6 ppm with J value of 8.0 Hz. For the 5-haloimidazole α -ribonucleosides in general, the anomeric protons appeared at 6.2–6.5 ppm (Fig. 9) and the 1'–2' coupling constant was close to 4.0 Hz (Table 11). All protected α -ribonucleosides have an NOE between the isopropylidene methyl protons and the imidazole C-2 proton, but the β -anomer does not have an NOE between these protons (Fig. 13). Examination of these ribonucleosides by high-resolution ^1H NMR (Tables 11 and 12) shows that the 5-haloimidazole ribonucleosides obey Imbach's rule in CDCl_3 and in CD_3OD . A strong NOE was observed between the H-5 imidazole proton and the anomeric proton in imidazole α -ribonucleoside while the 5-haloimidazole ribonucleosides do not have an NOE between these protons (Figs. 14 and 15), which strongly supports the structure of the 5-bromoimidazole and 5-iodoimidazole α -ribonucleosides.

The structure and anomeric configuration of all these α -ribonucleosides were established by 2D NMR spectroscopy (COSY, HMQC and NOESY). All protected α -ribonucleosides have an NOE between the isopropylidene methyl protons and the imidazole proton, whereas the β -anomer does not have an NOE between these protons (Fig. 14). Imbach's rule suggests that $0.18 < \Delta\delta < 0.23$ for the β -anomers and $0 < \Delta\delta < 0.10$ for α -anomers, where $\Delta\delta$ is the difference in chemical shift between the two methyl groups in the

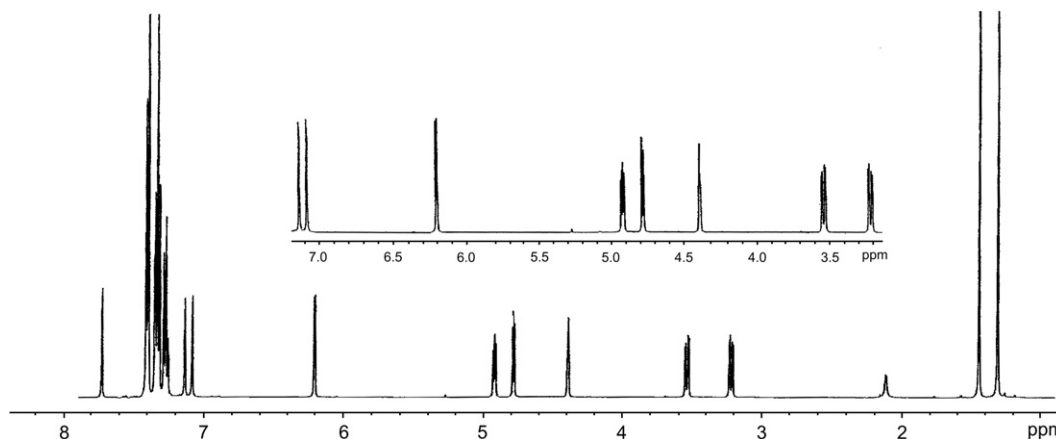


Figure 9. ^1H NMR of α -imidazole ribonucleoside.

isopropylidene-protected ribonucleosides. For the imidazole and benzimidazole α -ribonucleosides, $\Delta\delta$ falls in the range of 0.011–0.174 ppm, while the β -ribonucleosides fall in the narrow range of $0.234 \leq \Delta\delta \leq 0.27$ ppm (Fig. 12). The use of different solvents does not influence the applicability of the rule itself (Table 12). The methyl protons of the isopropylidene had an NOE with the imidazole proton in 5'-deprotected α -benzimidazole ribonucleoside **124** (Scheme 24) but the β -benzimidazole nucleoside does not have an NOE between those protons (2D NOESY). Structural proof for the α -anomer was also confirmed with the help of NOE difference spectroscopy. Irradiation of the isopropylidene methyl protons of 5'-deprotected benzimidazole ribonucleoside **124** caused a positive 10% NOE enhancement of the imidazole proton, and its precursor **55** (5'-protected ribonucleoside) shows a 12% NOE enhancement upon irradiation of the methyl proton. The signals for the methyl protons of the isopropylidene in the benzimidazole ribonucleoside were visible at δ 1.279 and 1.382 ppm ($\Delta\delta=0.103$). The anomeric proton signal appeared at δ 6.337 ppm with a J value of 4.5 Hz, which further confirms the α -configuration of the desired ribonucleosides.

5.3. NMR and X-ray structures of indoline and indole α -ribonucleosides

The anomeric protons of the α -indole ribonucleosides in NMR appeared at δ 5.32–5.45 ppm and the 1'-2' coupling constant was 3.8 Hz for each compound. For comparison, the protected α - and β -ribonucleoside of benzimidazole and 5,6-dimethylbenzimidazole had $J_{1'-2'}=3.8$ Hz for the α -anomers, but 3.0 or 3.2 Hz for the β -anomers. While literature reports suggest that the anomeric configuration of such nucleosides can be established based on the chemical shift difference of the isopropylidene methyl groups, the indoline ribonucleosides do not follow this rule. Thus, while the benzimidazole α -ribosides have isopropylidene methyl group chemical shift separations of ≤ 0.2 ppm (0.16 and 0.20 ppm) and the β -ribosides have a 0.27 ppm methyl separation, the α -indoline ribosides have an isopropylidene separation of 0.22 or 0.23 ppm.

The anomeric configuration of the indoline nucleoside, **79**, was confirmed by X-ray crystallography (Fig. 16). Suitable crystals for X-ray diffraction were grown by slow

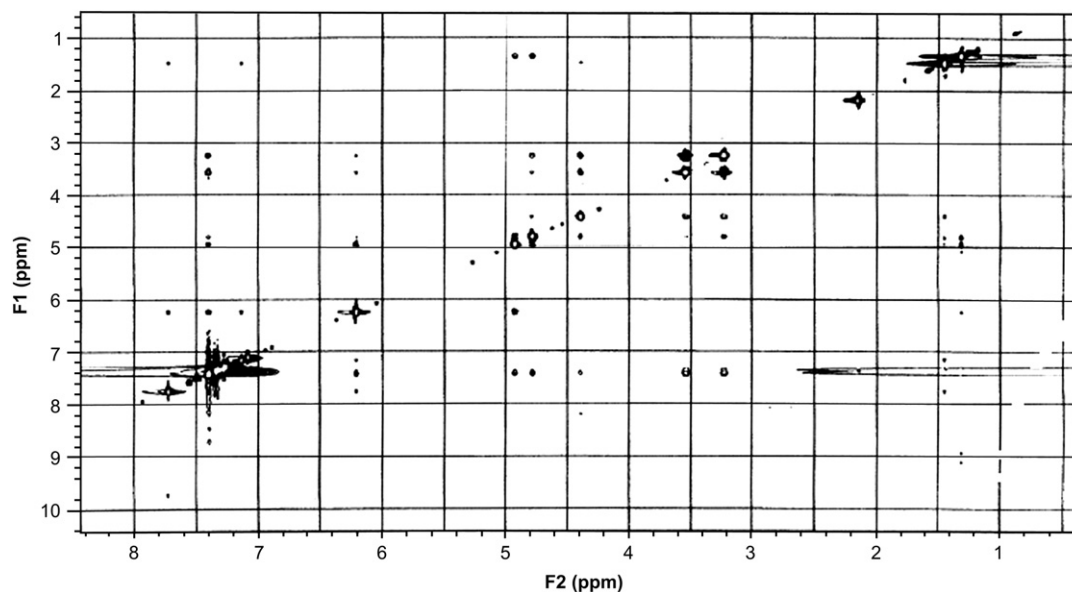


Figure 10. NOE of 1-(5'-*O*-triphenylmethyl-2',3'-*O*-isopropylidene- α -D-ribofuranosyl)imidazole.

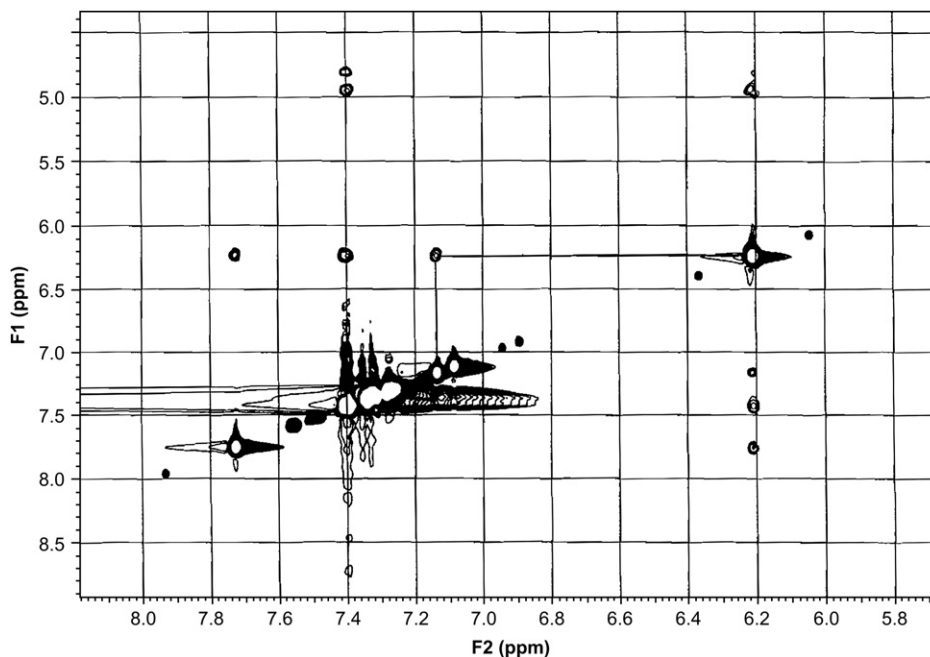


Figure 11. Downfield region NOE of α -imidazole ribonucleoside showing NOE between anomeric proton and C-5 imidazole proton.

crystallization at low temperature in ethyl acetate/hexane (1:1). The indoline moiety is nearly planar, the glycosidic bond length is 1.444 Å, and the glycosidic torsion angle, C(19)–N(1)–C(1)–O(1) is -79.9° , slightly outside the narrow range of -30° to -72° observed for other α -ribonucleosides.

5.4. Determination of anomeric configuration by cross-correlated relaxation between $H^{1'}$ chemical shift anisotropy and $H^{1'}-H^{2'}$ dipolar relaxation mechanisms modulated by local motions in ribonucleosides

More recently, we investigated the role of internal motions on $H^{1'}-H^{2'}$ dipolar and $H^{1'}$ chemical shift anisotropy

(CSA) cross-correlated NMR relaxation measurements on various α/β -ribonucleosides as model compounds, and the use of cross-correlated NMR relaxation as a direct and convenient method to determine the anomeric configuration of ribonucleosides.¹⁰ We observed that the existence of large amplitude internal motions in the ribonucleosides due to glycosidic C–N bond rotation and pseudorotation in the ribose moiety modulate the $H^{1'}-H^{2'}$ dipolar–CSA ($H^{1'}$) interaction^{84–96} in a distinct way, so that nucleosides in the α -configuration show significant and detectable cross-correlation effects in the proton nuclear spin-lattice relaxation, whereas no such effects are observed for β -nucleosides. The cross-correlation effects can be observed using the conventional inversion-recovery experiment that is used to measure the

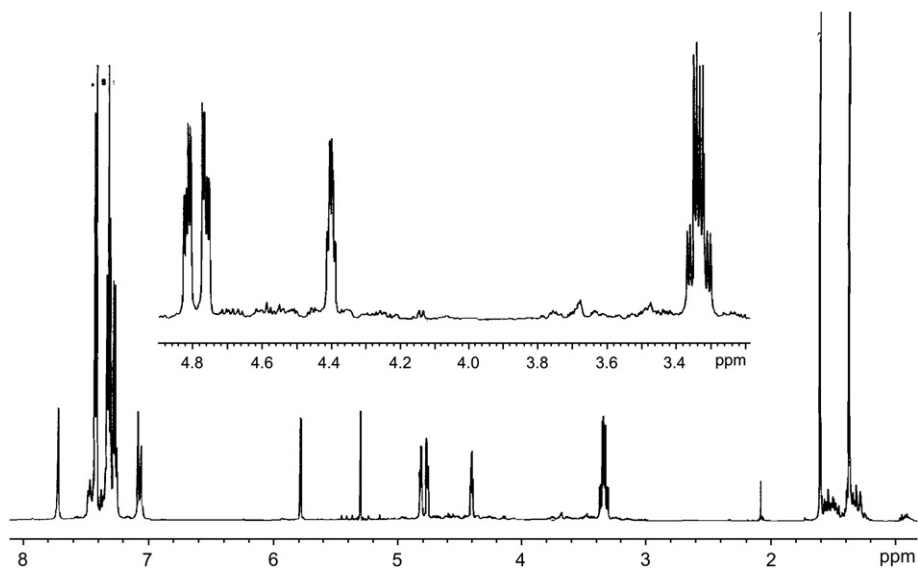


Figure 12. ^1H NMR of β -imidazole ribonucleoside.

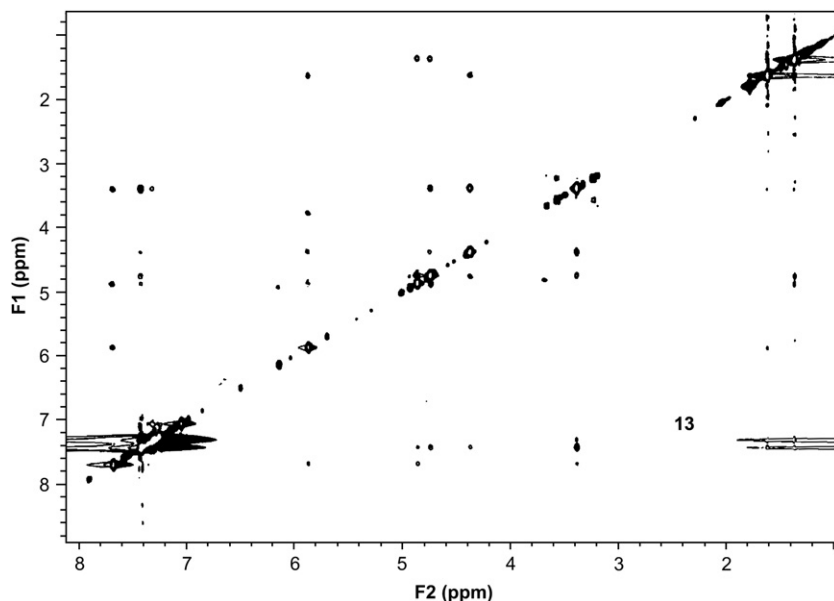
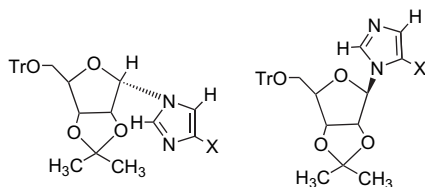


Figure 13. NOE of 1-(5'-*O*-triphenylmethyl-2',3'-*O*-isopropylidene- β -D-ribofuranosyl)-5-bromoimidazole.

Table 12. Methyl proton separation (Δ in ppm) for α/β -imidazole/benzimidazole ribonucleosides



S. No.	Compound	Me ₁ (δ ppm)	Me ₂ (δ ppm)	Δ (ppm)	Solvent
1	α -5-Fluoroimidazole	1.318	1.406	0.088	CDCl ₃
2	α -5-Bromoimidazole	1.301	1.394	0.093	CDCl ₃
3	α -5-Iodoimidazole	1.29	1.39	0.10	CDCl ₃
4	α -5-Fluoroimidazole	1.296	1.307	0.011	CD ₃ OD
5	α -5-Bromoimidazole	1.390	1.416	0.026	CD ₃ OD
6	α -5-Iodoimidazole	1.280	1.356	0.076	CD ₃ OD
7	α -5-Fluoroimidazole	1.277	1.310	0.033	DMSO- <i>d</i> ₆
8	α -5-Bromoimidazole	1.246	1.340	0.094	DMSO- <i>d</i> ₆
9	α -5-Iodoimidazole	1.297	1.250	0.047	DMSO- <i>d</i> ₆
10	β -5-Fluoroimidazole	1.359	1.586	0.22	CDCl ₃
11	β -5-Bromoimidazole	1.370	1.617	0.24	CDCl ₃
12	β -5-Fluoroimidazole	1.345	1.546	0.20	CD ₃ OD
13	β -5-Bromoimidazole	1.339	1.556	0.21	CD ₃ OD
14	α -Benzimidazole	1.313	1.447	0.134	CDCl ₃
15	α -Benzimidazole	1.299	1.437	0.138	CD ₂ Cl ₂
16	α -Benzimidazole	1.279	1.412	0.133	Acetone- <i>d</i> ₆
17	α -Benzimidazole	1.282	1.358	0.076	CD ₃ OD
18	α -Benzimidazole	1.246	1.340	0.094	DMSO- <i>d</i> ₆
19	β -Benzimidazole	1.390	1.658	0.268	CDCl ₃
20	α -Imidazole	1.312	1.447	0.135	CDCl ₃
21	α -Imidazole	1.280	1.356	0.076	CD ₃ OD
22	α -Imidazole	1.225	1.377	0.15	DMSO- <i>d</i> ₆
23	β -Imidazole	1.367	1.601	0.234	CDCl ₃
24	β -Imidazole	1.339	1.548	0.209	CD ₃ OD
25	β -Imidazole	1.301	1.509	0.208	DMSO- <i>d</i> ₆
26	α -5,6-Dimethylbenzimidazole	1.328	1.502	0.174	CDCl ₃
27	β -5,6-Dimethylbenzimidazole	1.401	1.672	0.271	CDCl ₃
28	α -Benzimidazole-5-OH	1.268	1.371	0.103	CDCl ₃
29	α -Benzimidazole-5-OH	1.268	1.293	0.025	CD ₃ OD
30	β -Benzimidazole-5-OH	1.403	1.672	0.27	CDCl ₃
31	β -Benzimidazole-5-OH	1.380	1.627	0.25	CD ₃ OD
32	β -Benzimidazole-5-OH	1.343	1.582	0.25	DMSO- <i>d</i> ₆

spin-lattice relaxation time, but with a small flip angle mixing pulse since with the 90° mixing pulse used in the conventional experiment, two spin-order is converted into multiple quantum coherence and cannot be observed. The difference between the intensities of the doublet components is directly related to the cross-correlation rate ($\Delta_{1',1'-2'}$) by which the two spin-order is created due to the H^{1'} CSA and H_{1'}-H_{2'} dipolar relaxation mechanisms. The spectra showing the relaxation behavior at different mixing times in the inversion-recovery experiment for the H^{1'} proton doublet of the α - and β -dimethylbenzimidazole are shown in Figure 17. The amount of the H_zH_{z'} created due to dipole-CSA cross-correlations between H^{1'}-H^{2'} and H^{1'} CSA is given in (Table 13). The H_{z0}, the equilibrium magnetization of H^{1'} spin, is calculated as the sum of the intensities of the H^{1'} doublet and the two spin-order is normalized with respect to it. The values of the normalized two spin-order clearly show that there is no effect of dipole-CSA cross-correlations on the ribonucleosides in the β -anomeric configuration.

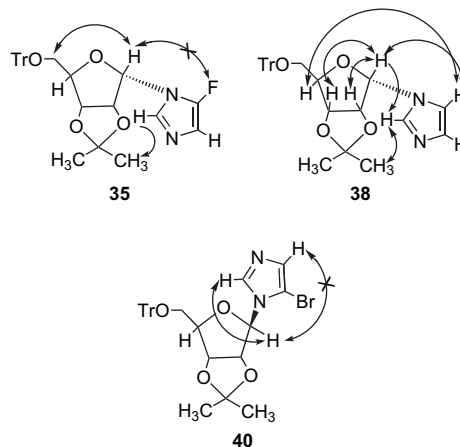


Figure 14. NOEs of α/β -5-haloimidazole ribonucleosides.

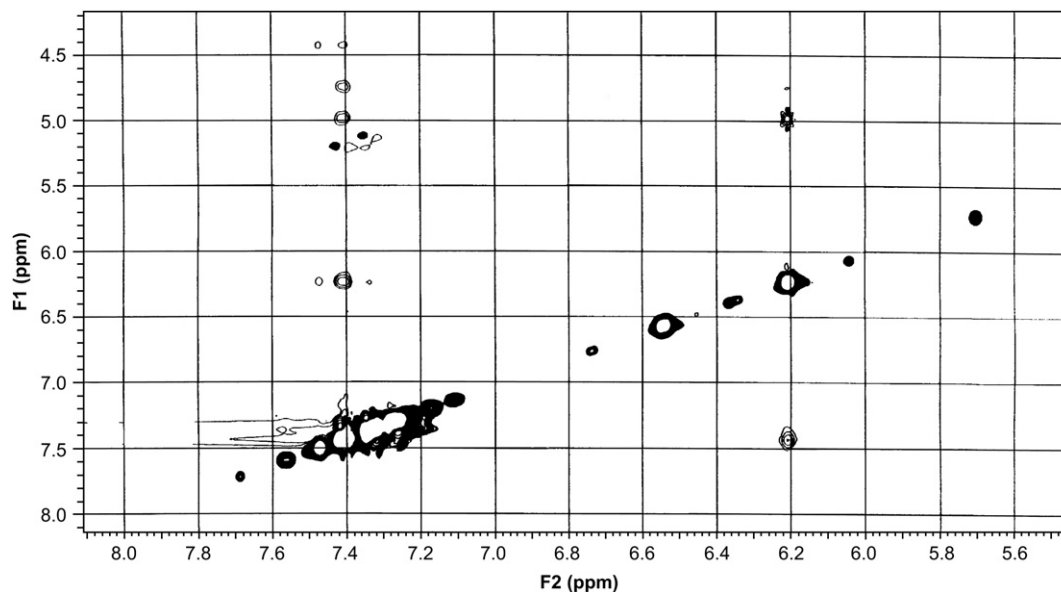


Figure 15. Downfield region NOE of 1-(5'-O-triphenylmethyl-2',3'-O-isopropylidene- α -D-ribofuranosyl)-5-fluoroimidazole.

The internuclear distance for the α - and β -anomer of similar ribonucleosides is known from their X-ray structures and the estimated ratio of $(d_{1'2'})^\alpha/(d_{1'2'})^\beta$ is 2.83 providing one of the reasons why cross-correlation effects are seen to be stronger for α -ribonucleosides. However, the dipole–CSA cross-correlation rate also depends on the magnitude and the orientation of the $H^{1'}$ CSA tensor. But the orientation of the tensor largely depends on the geometry of the molecule, and the crystal structure for similar ribonucleosides suggests that it may be close to the magic angle for the β -anomer, which also makes the dipole–CSA cross-correlation smaller. It is known that the sugar moieties in nucleosides are involved in a dynamically interconverting two state conformational equilibrium, C(2')-*exo* and C(3')-*endo* (N type) and C(2')-*endo* and C(3')-*exo* (S type), which occurs in the nanosecond time scale at room temperature.⁹⁷ Also, C–N glycosidic bond rotation is known to occur in the nanosecond time scale.⁹⁸ We performed the inversion-recovery experiments

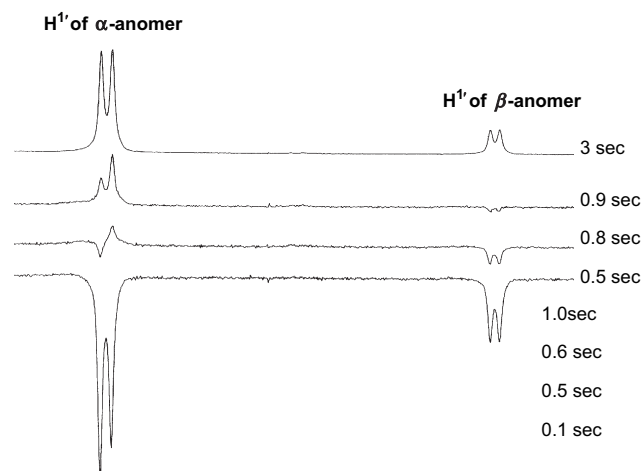


Figure 17. ^1H inversion-recovery spectra of the mixture of **6** and **6b** (80 and 20%) for different mixing times. The $H^{1'}$ differential recovery for the α -anomer indicates the presence of dipole–CSA cross-correlations whereas the β -anomer shows no such effect. $H^{1'}$ chemical shift and $J_{H^{1'}-H^{2'}}$ values of **6** and **6b** are 6.48 ppm, 4.95 Hz and 6.13 ppm, 4.24 Hz, respectively. Experiments were done on an INOVA 500 MHz Varian NMR spectrometer at 298 K. The sample was dissolved in deuterated CD_3OD .

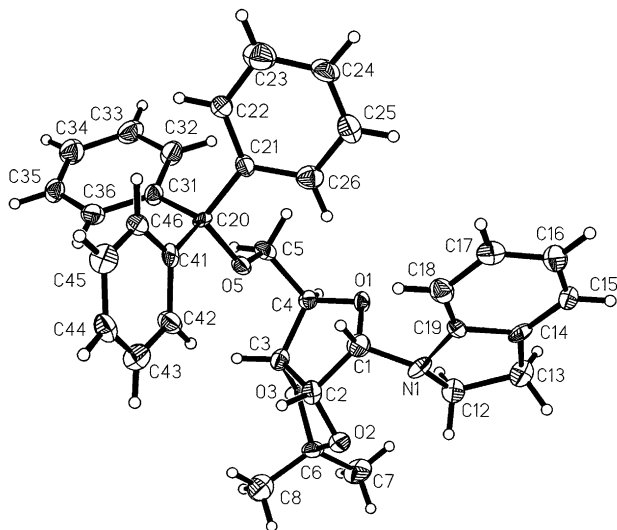


Figure 16. ORTEP diagram of compound α -indoline ribonucleoside **79**.

on the α -benzimidazole ribonucleoside in 30% glycerol, which led us to the conclusion that ^1H – ^1H dipolar and ^1H CSA cross-correlated relaxation rates are dominated by these two large amplitude internal motions rather than the

Table 13. Normalized proton–proton two spin-order values for the α - and β -ribonucleoside

Ribonucleosides	Anomeric configuration	$(H_z^1 H_z^{2'}) / (H_{20}^1) \times 100$
α -Benzimidazole	α	9.3
β -Benzimidazole	β	None
α -Dimethylbenzimidazole (6a)	α	7.06
β -Dimethylbenzimidazole (6b)	β	None
α -Imidazole	α	5.89
β -Imidazole	β	None

overall molecular motion and therefore contribute significantly to longitudinal cross-correlation rates for α -ribonucleosides but not for β -ribonucleosides, where such motions may be lesser in magnitude. It is important to note that longitudinal cross-correlations are more sensitive to faster molecular motions due to the fact that they affect only the single quantum transition probabilities (in the case of weakly coupled spin systems). Therefore, it may be argued that α -ribonucleosides are less rigid in comparison to their β -anomeric counterparts where we do not see any cross-correlation effects. It is known that contributions from polypeptide backbone fluctuations to the conformational entropy and heat capacity of globular proteins result in higher thermal stability of these proteins. In a similar fashion, the internal motions present in the α -ribose moiety could contribute to the conformational entropy/heat capacity of the rarely found α -ribonucleosides both in their free form and in the structure of vitamin B₁₂ and nicotinamide adenine dinucleotide, thus enhancing their structural and thermodynamic conformational stabilities. Ab initio and quantum chemical studies to investigate the H¹ CSA dependence on the sugar pucker and glycosidic bond rotation, solid state NMR studies to estimate the ¹H CSA tensor components, and more relaxation studies using anisotropic motional models would be needed to substantiate the exact reasons for our experimental observations.

6. Conclusions

Vitamin B₁₂ and its co-enzyme analogs contain a ribonucleoside lower axial ligand in the biologically unusual α -configuration as part of corrin ring appendage called the nucleotide loop. In order to determine if this axial ligand is involved in the enzymatic functioning of the B₁₂ co-enzyme, 5-deoxyadenosylcobalamin, we have pursued analogs in which the axial ligand is structurally altered. These include imidazole and substituted imidazole α -ribonucleosides, which permit the determination of the effect of axial nucleotide steric bulk and basicity on the co-enzymatic function of co-enzyme B₁₂, as well as α -indole nucleosides, which can be used to test the importance of a coordinated axial nitrogen in the B₁₂ dependent enzymatic reactions. While B₁₂ analogs with altered axial nucleotides can be obtained by 'guided biosynthesis', using fermentation of *P. shermanii* on media supplemented with the desired axial base, the scope of this method is limited in that some imidazole and benzimidazole analogs are incorporated poorly, and indoles are not incorporated at all, evidently due to their lack of activity with the necessary ribosyltransferase enzyme, which catalyzes the formation of *N*-glycosidic bond. Such derivatives are best prepared by semisynthesis, coupling cobyrinic acid mixed anhydride with the 3-phosphodiester of the desired ribonucleoside with (*R*)-(-)-1-amino-2-propanol (Scheme 1). Consequently, the preparation of such cobalamin analogs requires robust methods for the synthesis of α -ribonucleosides. As most naturally occurring ribonucleosides have the β -anomeric configuration, most of the synthetic effort to date has been targeted at the creation of the β -*N*-glycosidic bond. Here, we have explored the utility, scope, and permutations of existing and new methods of *N*-glycosidic bond formation and for the protection and deprotection of the α -ribonucleosides to enable the

semisynthesis of a wide variety of B₁₂ analogs with altered axial nucleoside ligands.

References and notes

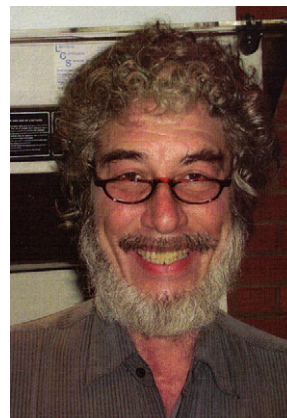
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