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Vitamin B_{12} and α-Ribonucleosides

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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 B_{12} 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 B_{12} dependent ribonucleoside triphosphate reductase,[3] ethanolamine amamonia lyase,[4] glutamate mutase[5] and methylmalonyl CoA mutase require a B_{12} cofactor. The cofactor for these reactions, 5- deoxyadenosylcobalamin (AdoCbl, coenzyme B_{12}, 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 10^9 to 10^{14}-fold remains unknown.

Vitamin B_{12} 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"), and various upper axial ligands (cyanide = vitamin B_{12}, CH_3 = 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 B_{12} coenzyme. 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 B_{12} for mechanistic studies.

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 the activation of co-enzyme B_{12}.[1] A number of nucleoside and oligonucleotide analogues have been used in medicinal applications due to their diverse biological effects human beings,[16] but the majority of these compounds are analogues 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 analogues is limited due to limited availability of synthetic routes to these anomers. Thus, only a limited number of α-nucleosides are known for their biological activity

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in human beings. α-2-Deoxythioguanosine has shown promising antitumer 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 produces up to 40–50% of the α-anomer,[15] it is often difficult to separate the α-anomer from its *β*-counterpart by column chromatography or crystallization, due to their similar Rf values and chemical nature. Since indoles and indolines are not incorporated in to 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-dimethybenzimidazole nucleotide[18] with a 5,6-dimethyindole nucleotide loop will produce an exact replica of the cobalamin without a coordinating nitrogen and serve as a test of 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 vechicles 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).[19 (b–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 similar to that of CNCbl-e-monocarboxylate. 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 analogues.

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 enzymetic and non-enzymetic 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]

2. Vitamin B_{12} and α-ribonucleosides

Vitamin B_{12} has attracted considerable attention since its discovery as the antipernicious anemia factor in liver.[24] The 5,6-dimethyl-α-D-ribofuranosylbenzimidazole of cobamins 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 semi synthesis/partial synthesis of cobalamin analogs[25] 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 analogues of B_{12}[1,2] in which the lower axial ligand is replaced with benzimidazole, imidazole, 4/5-fluoroimidazole and 4/5-bromoimidazole and 4/5-methylimidazole.[1] These analogues 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 B_{12} analogues 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 analogues, 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 *Propionibacterium shermanii* on media supplemented with desired base (Table 1), employing a modification of the method of Renz.[26] *P. shermanii* (ATC 13673) was grown on solid micro assay culture agar (DIFCO) in a test tube and made anaerobic by overlaying the culture with unsolidified, sterilized agar solution. After 5 days of growth at 30 °C, the culture was stored at 4 °C until use. The desired bases, were added to the fermentation media as an ethanolic solution containing 20–30 mg. After the usual workup the crude biosynthetic product was loaded onto a 4 × 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 × 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 B_{12} 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 coenzyme B_{12} has been investigated using the coenzyme 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-ribosyl moiety and the bulky axial ligand of the nucleotide loop of adenosylcobalamin in coenzymic function have been investigated using two series of coenzyme 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-dimethyl benzimidazole base and a trimethylene spacer group in the case of the imidazole base (Fig 6). The authors found that these analogues 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 coenzyme, and this interest has only been heightened by recent findings that different AdoCbl-dependent enzymes manipulate the axial ligation of the coenzyme 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.

2.3. Semisynthesis of vitamin B_{12}, and Synthesis of 5,6-dimethyl benzimidazole α-ribonucleotide loop

Woodward and Eschenmoser took on the enormous task of the total synthesis of vitamin B_{12}, a complex and sensitive biomolecule. The synthesis of the vitamin B_{12} has been characterized as the most spectacular synthetic endeavour of the 20th century.[32] Cobyric acid is the primary target of vitamin B_{12} synthesis (Scheme 1), and can be used to preparing cobalamin derivatives modified at lower axial ligand. Acid hydrolysis of vitamin B_{12} either by hydrochloric acid or TFA, produces cobyric acid in moderate yields.[33] In order to synthesize the analogues of cobalamins modified at lower axial ligand, this acid hydrolysis of vitamin B_{12} remains the best option.

In 1952, Folkers and co-workers reported for the first time degradation products of B_{12} by acid hydrolysis and they later compared the properties of the isolated product with synthetic α-ribozole (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-ribozole, **6** (Scheme 2).

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-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 two molar equiv 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-Ribozole **6** was easily obtained by catalytic debenzylation using 10% palladim 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-dimethylbenimidazole in acetonitrile decreases the yield to 25%. The synthetic scheme for B_{12} is shown in scheme 4, starting from the protected 5,6-dimethylbenzimiodazole ribonucleoside, **9**. The nucleotide loop was prepared in seven steps. Cobyric acid mixed anhydride was condensed with the nucleotide loop in the presence of base. D-1-(Benzyloxycarbonylamino)propan-2-ol can also be condensed with cobyric acid in presence of acid (Scheme 5) and gives two isomeric phosphodiesters which can be easily separated by chromatography. The reaction of the mixture of the 2′- and 3′-phosphodiester with cobyric acid afforded the corresponding 2′ and 3′ isomers of cobalamin. The 2′ isomer was found to be inactive and more labile compared to B_{12} due to steric effects.[33(d)]

Norvitamin B_{12}, **17** is the first known naturally occurring B_{12} cofactor which lacks the peripheral methyl group in the propanol residue of the nucleotide loop. Krautler and co-workers[36] prepared it by partial synthesis from cobyric acid and 2-aminoethyl-3-ribazolyl phosphate in 73% overall yields (Scheme 6). The nucleotide loop was synthesized converting

ethanolamine to its cyclic phosphate. The reaction produces the two isomeric 3′- and 2′-phosphates, 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. 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 etherate[43] 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 glycosylation[44] method is now frequently used for 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-aceloxy groups give *N*-glycosides with the *β*-configuration due to neighboring group participation.

Fletcher and co-workers[35] utilized partially benzylated sugars for making α-ribonucleosides. Condensation of 2′,3′,5′-tri-*O*-benzyl-D-ribofuranosyl chloride with 5,6-dimethylbenzimidazole produces up to 66% protected ribonucleoside and subsequent catalytic deprotection gives α-ribozole 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.

Mukaiyama and co-workers[15] showed that the reaction of 1-hydroxy sugars such as 2,3-*O*-(1-methylethylidene)-5-*O*-(triphenylmethyl)-α/*β*-D-ribofuranose[45] or 5-*O*-benzoyl-2,3-*O*-(1-methylethylidene)-D-ribofuranose[46] with trimethylsylated benzimidazole and other nitrogenous bases, including nucleoside bases and azides, using 2-fluoro-1-methylpyridinium tosylate as condensing reagent (Scheme 8), provides predominantly alpha 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 has been reported. There are reports[47] of the use of ribofuranosyl chlorides for α-glycosylation, but these also produce mixtures of α- and *β*-*N*-glycosides. There had been not a single report of a reaction which produces the α-nucleoside exclusively (Table 2) until our report[12] that the reaction of TMS protected indoline bases (dimethyl indoline, 5-bromo indoline 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. The 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 towards nucleoside bases then 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 produces the 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, a series of α-ribonucleosides of 5-haloimidazole we prepared 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. 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*-anomers of 5-haloimidazole nucleosides, and no 4- halo substituted imidazole was detected.

There are reports of trimethylsilyltriflate-catalyzed glycosylation of 4(5)-substituted imidazole with protected ribose, which produces both 4- and 5-substituted *β*-imidazole ribosides 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 ^{1}H NMR of crude reaction mixtures showed only two products, the α-(major) and *β*-anomer (minor) of the 5-haloimidazole ribonucleoside, as established using ^{1}H nOe after purification.

Interestingly, when 4(5)-fluoroimidazole was used to supplement the growth medium during the fermentation of *P. shermanii*, two analogues of cyanocobalamin were produced, one with 4-fluoroimidazole axial nucleotide and one with 5-fluoroimidazole axial nucleotide (Scheme 11). These two analogues were distinguished by the prominent ^{1}H NMR nOe crosspeak between the imidazole 5-H and the ribose anomeric proton in the 4-flurorimidazole analog, which is absent in the ROESY spectrum of 5-fluoroimidazole analogue. The structures of these isomers were confirmed by complete ^{1}H and ^{13}C NMR assignments and mass spectroscopy. For further study, 4-fluoro imidazole α-ribonucleoside analogues can be made by the hydrolysis of corresponding cyanocobalamin analogues.

For spectroscopic comparison the α- and *β*-ribonucleosides of imidazole were 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 hr. 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 hr. 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. Semi synthesis of Co*β*-cyanobenzimidazolylcobamide—We successfully prepared Co*β*-cyanobenzimidazolylcobamide from α-benzimidazole ribonucleoside.[65] The α-riboucleotide 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 cobyric acid mixed anhydride obtained from vitamin B_{12} hydrolysis. The α-ribonucleoside was isolated in pure form as a crystalline solid in 70% yield by fractional crystallization using an ethylacetate:hexane mixture. The protected nucleoside was deprotected without any difficulty using trifuoroacetic acid at ambient temperature or catalytic carbon tetrabromide in methanol in excellent yield. The deprotected ribonucleoside **56** was selectively protected at 5′ with trityl chloride in pyridine at ambient temperature. Phosphoryation 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′ nucleotides. Without any further purification, the nucleotide mixture was converted to the corresponding 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 (**62**) and the 2-isomer (**63**) in 70:30 ratio after reverse phase chromatography. Cobyric acid was converted to the mixed anhydride for final coupling with nucleotide loop. The semisynthesis/partial synthesis of B3-deazacobalamin (vitamin B_{12} derivatives, Fig. 1) can also achieved from 5,6-dimethylindoline α-ribonucleoside (Scheme 13). The α-indoline nucleoside was converted to the indole ribonucleoside using MnO_2 in presence of type 4Å molecular sieves. Subsequent deprotection of the ribonucleoside and phosphorylation were easily completed by the procedures in scheme 13. Further synthesis of the nucleotide loop is in progress.

3.1.3. Indoline α-Ribonucleosides—We reported 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 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 method[66] starting from the readily available 5-nitropseudocumene, **67**.[67] 5-Nitropseudocumene 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 dimethyindoline, **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-trimethylsilanyl-2,3-dihydro-1H-indole, **77**, was prepared in 98 % yield from dimethylindoline at −70 °C. Similarly, the TMS-bromoindoline, **78**, was prepared in 95 % yield from corresponding 5-bromoindoline base.

The silylated dimethyindoline 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*-anomers), 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 hr. 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 prepared 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 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 4Å 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 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. The structure of these indoline ribonucleosides prepared by direct glycosylation was also confirmed by ^{1}H NMR, ^{13}C NMR and as well as 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 reported for the indoline ribonucleosides[8] prepared by the 2-fluoro-1-methylpiridinium 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 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 ^{1}H NMR. For purification purposes, the uracil and cytosine α-ribonucleosides were deprotected at 5′ in order to separate the corresponding nucleosides from their *β*-counterparts and were isolated in 63 and 30% respectively.

The 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 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′ 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.[10] For the semisynthesis of cobalamins[1] (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 of the dehydrogenation of the related *β*-indoline nucleosides to corresponding *β*-indole nucleosides. Preobrazhenskaya[70] 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 hr. 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 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).

1-(5-O-triphenylmethyl-2,3-*O*-isopropylidene-α-D-ribofuranosyl)indoline, **79**, was dehydrogenated in either benzene or methylene chloride using MnO_2 in presence of type 4Å 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 one hour in benzene at 40–50 °C whereas the 5-bromoindoline, **81**, takes 1.5–3.0 hr (Table 6). After completion, the reaction mixture was cooled to room temperature and filtered through celite, and after the usual work up, 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 MnO_2 and molecular sieves and proceeded smoothly without any decomposition, in

95% yield (dimethylindoline). The reaction, however proceeds much faster (1–1.5 hr) in benzene than 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 ^{1}H NMR, ^{13}C NMR, high resolution mass spectroscopy and as well as 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** in 50% overall yields.[72] Thymidine protected with diphenyl 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. In particular, formation of an intramolecularly cyclized iminium ion from the oxonium ion intermediate, due to the neighboring group participation by the diethylthiocarbamoyl group, tended to decrease the overall reaction rate. The α/*β* C1′ epimerization could be carried out with an α-anomer selectivity of 89% using the $Et_2CHC(O)$ group. Thus, 5′-*O*-pixyl-α-thymidine could be synthesized from *β*-thymidine as a key intermediate for the synthesis of α-DNA in a considerably improved overall yield of 40%.

3.7 Boron trichloride complex method

Furukawa and co-workers used the BCl_3 complex of ribose **111** to prepare α-adenosine[48] **112** (scheme 21). Octanoyladenine and the sugar were heated for 3 hr 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.

3.8 Enzymetic separation of α/*β* - nucleosides

Quite recently, the enzyme lipase was used to separate mixtures of α and *β*-deoxyribonucleosides 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 **113** & **114**. *Pseudomonas cepacia* (PSL-C), showed excellent regioselectivity for the hydrolysis of 5′-*p*-chlorobenzoate in case of α-thymidine, while it was inactive with *β*-thymidine. After hydrolysis of thymidine, the mixure 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 the protection of the 5-hydroxyl and 2,3-hydroxyl groups in both carbohydrate and nucleoside chemistry.[74] For α-ribonucleosides, protection of the 2′, 3′ and 5′ positions 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 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, dimethyindole, 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 describe 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 ribonucleoside (**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-ribonucleoside (**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.

The 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-ribonucleosides were easily deprotected at the 5′- and 2′, 3′-positions without any deglycosylation using a mixture of TFA:H_2O (1:1) at ambient temperature.

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 CBr_4 in methanol at reflux under neutral reaction conditions,[77] at these temperatures, imidazole ribonucleosides isomerize from the α- 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 photoirradation using catalytic carbon tetrabromide (CBr_4) in methanol at ambient temperature. Although selective deprotection of trityl *vs* isopropylidene groups of *β*-ribonucleodsides and sugars has been achieved using photolysis,[81] our photolysis protocol shows no selectivity in trityl/isopropylidene deprotection for the α-ribonucleosides.

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 hr at ambient temperature. Complete conversion of the starting material was confirmed by TLC (methanol: methylene chloride 8:2) as well as ^{1}H NMR. Increasing the amount of carbon tetrabromide from 0.05 to 0.5 equivalent 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.

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

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 ^{1}H and ^{13}C NMR spectroscopy. Due to the inclusion of α-ribazole in B_{12}, several NMR studies have appeared.[78] The structure of vitamin B_{12} and its analogues have been extensively studied by NMR and X-ray crystallography. The α-ribonucleoside in B_{12} 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 B_{12} 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 complexes by Marzilli and co- workers[79] 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 ^{13}C 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 B_{12}, the ribose 3′ proton and carbon chemical shifts of the α-ribazole moiety are altered in the ^{1}H NMR and ^{13}C NMR, because the ribose C3 is directly attached to phosphodiester linkage. In vitamin B_{12}, 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.

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] Imbach[83] suggests that the difference in the ^{1}H 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 $0.18<\Delta\delta<0.23$ for the *β*-anomers and $0<\Delta\delta<0.10$ 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) then 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 the 5-fluoroimidazole ribonucleoside (Fig. 8), the anomeric proton and the fluorine atom are separated by 2.386 Å in space. A similar distance 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 & 11). In the proton NMR, a doublet was observed at 6.54 ppm for the imidazole H-4 ($^{3}J_{HF}$ = 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 a nOe between the isopropylidene methyl protons and the imidazole C-2 proton, but the *β*-anomer does not have a nOe between these protons (Fig 13). Examination of these ribonucleosides by high resolution ^{1}H NMR (Table 11&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 a nOe between these protons (Fig 14), 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 a nOe between the isopropylidene methyl protons and the imidazole proton, whereas the *β*-anomer does not have a nOe between these protons. 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 Δδ is the difference in chemical shift between the two methyl groups in the isopropylidene-protected ribonucleosides. For the imidazole and benzimidazole α-ribonucleosides, Δδ falls in the range of 0.011–0.174 ppm, while the *β*-ribonucleosides fall in the narrow range of $0.234 \leq \Delta\delta$ 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 a nOe with the imidazole proton in 5′-deprotected α-benzimidazole ribonucleoside **124** (Scheme 24) but the *β*-benzimidazole nucleoside does not have a nOe between those protons (2D NOESY). Structural proof for the α-anomer was also confirmed with the help of nOe difference spectroscopy. Irridation of the isopropylidene methyl protons of 5′-deprotected benzimidazole ribonucleoside **124** caused a positive nOe enhancement 10% 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 (Δδ = 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*-ribonucleosides 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. 13). Suitable crystals for X-ray diffraction were grown by slow 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.[13] 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 used to measure the spin-lattice relaxation time, but with a small flip angle mixing pulse since sith 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 for the α- and *β*- dimethylbenzimidazole are shown in Figure 17. The amount of the $H_z^{1'}$ $H_z^{2'}$ created due to dipole-CSA cross-correlations between $H^{1'}$–$H^{2'}$ and $H^{1'}$ CSA is given in (Table 13). The $H_{z0}^{1'}$, 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 in the ribonucleosides in the *β*-anomeric configuration.

The internculear distance for the α- and *β*-anomers of similar ribonucleosides are 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 suggest 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 on 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 on the α-benzimidazole ribonucleoside in 30% glycerol which led us to the conclusion that ^{1}H-^{1}H dipolar and ^{1}H CSA cross correlated relaxation rates are dominated by these two large amplitude internal motions rather than the 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 results 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 as well as in the structure of vitamin B_{12} and nicotinamide adenine dinucleotide, thus enhancing their structural and thermodynamic conformational stability. Ab-initio and quantum chemical studies to investigate the $H^{1'}$ CSA dependence on the sugar pucker and glycosidic bond rotation, solid state NMR studies to estimate the ^{1}H CSA tensor components, and more relaxation studies using anisotropic motional models would be needed to substantiate the exact reasons for our experimental observations.

7. Conclusions

Vitamin B_{12} and its coenzyme 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 B_{12} coenzyme, 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 coenzymetic function of coenzyme B_{12}, as well as α-indole nucleosides which can be used to test the importance of a coordinated axial nitrogen in the B_{12} dependent enzymatic reactions. While B_{12} analogs with altered axial nucleotides can be obtained by "guided biosynthesis", using fermentation of *Propionibacterium shermanni* 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 catalyze the formation of *N*-glycosidic bond. Such derivatives are best prepared by semisynthesis, coupling cobyric 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 B_{12} analogs with altered axial nucleoside ligands.

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Biographies

Tilak Chandra was born in Pithoragarh, state of Uttaranchal, India. He completed his MS degree from Kumoun University & Ph.D. degree from Central Drug Research Institute (CDRI) Lucknow in 1995, where he worked on nucleoside syntheses as potential antiviral and antiallergenic activity. In 2002 he joined Prof. Kenneth L. Brown's research group at Ohio University as postdoctoral Scientist to work on the semi synthesis of B3-deaza cobalamins. Currently he is working in Prof. Broderick's research group and his research interests concentrate on the synthesis of nucleosides, nucleotides and oligonucleotides.

Kenneth L. Brown was born in Philadelphia, PA. He earned a BS in Biochemistry from the University of Chicago in 1968 and a Ph.D. in Biochemistry from the University of Pennsylvania in 1971. After working as a NIH Postdoctoral Fellow in the Department of Biochemistry and Biophysics at the University of California at Davis in Lloyd Ingraham’s laboratory, he joined the faculty in the Department of Chemistry at the University of Texas, Arlington, in 1975. In 1990 he moved to Mississippi State University as Professor and Head of the Department of Chemistry, and in 1996 he moved to Ohio University where he is now Professor of Chemistry and Biochemistry.

Abbreviations

B_{12}, vitamin B_{12}
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
TMSOTf, trimethylsilyl triflate
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

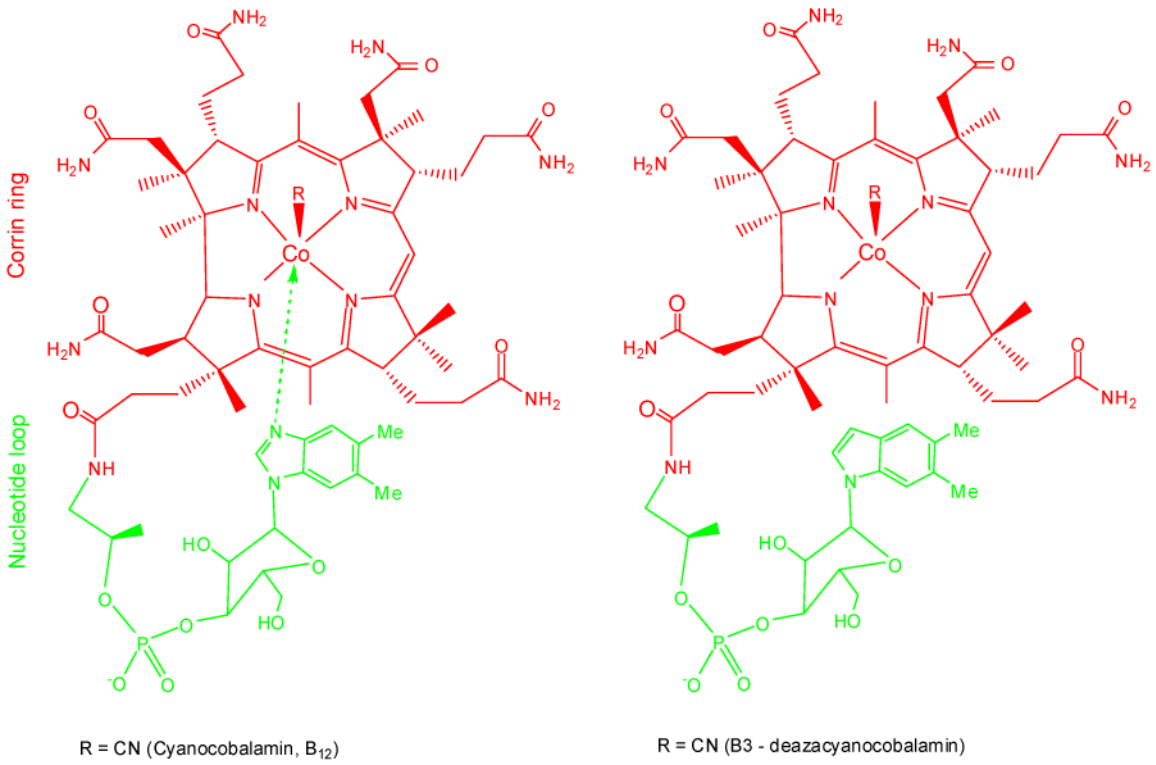


Figure 1.

Vitamin B_{12} derivatives with altered lower axial ligands are of interest as probes of the mechanism of action of coenzyme B_{12}. In order to determine what role, if any, the lower axial ligand plays in the enzymatic activation of coenzyme B_{12}, it is necessary to synthesize vitamin B_{12} derivatives with altered axial nucleosides. Although some such analogs may be obtained by guided biosynthesis,[7] using fermentation of appropriate bacteria on media supplemented with the desired axial base, analogs with indole axial nucleotides (B3-deazacobalamins, Fig. 1) which mimic the natural structure but lack the coordinating nitrogen cannot, as these organisms fail to glycosylate indoles to make the required nucleoside precursor.[8] Semi-synthesis of such cobalamin derivatives is best achieved by coupling cobyric 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]

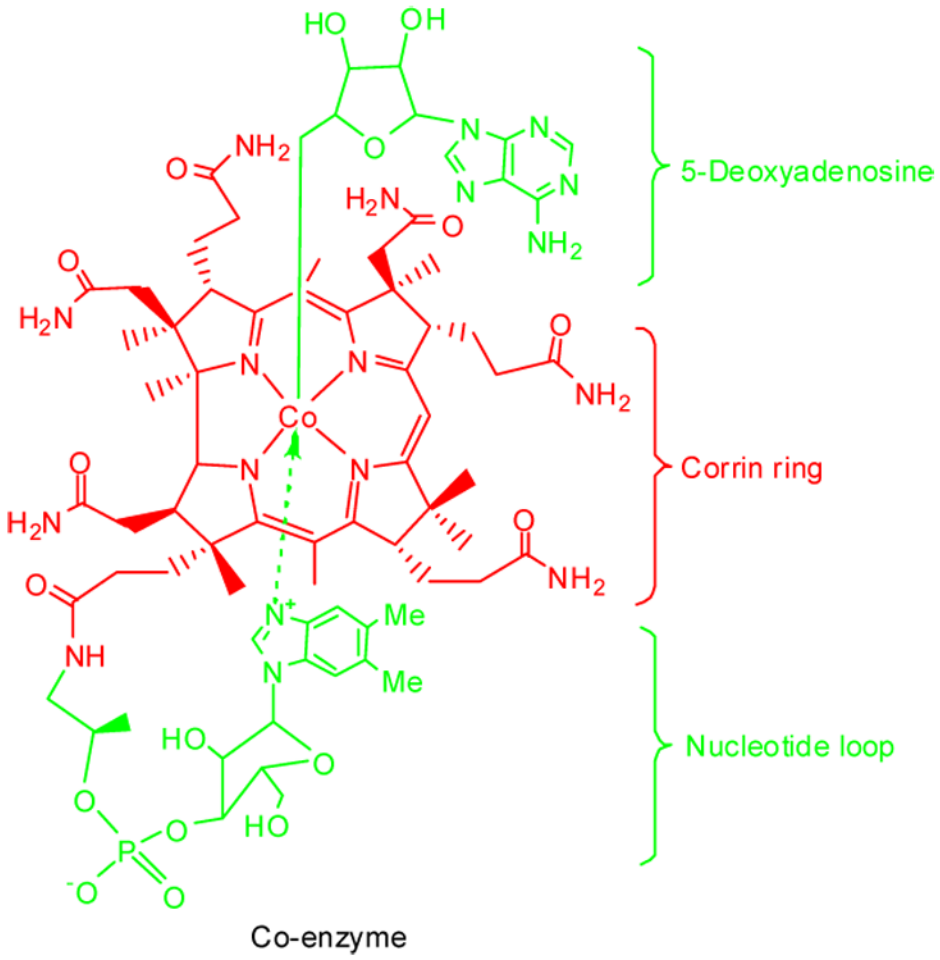


Fig. 2.

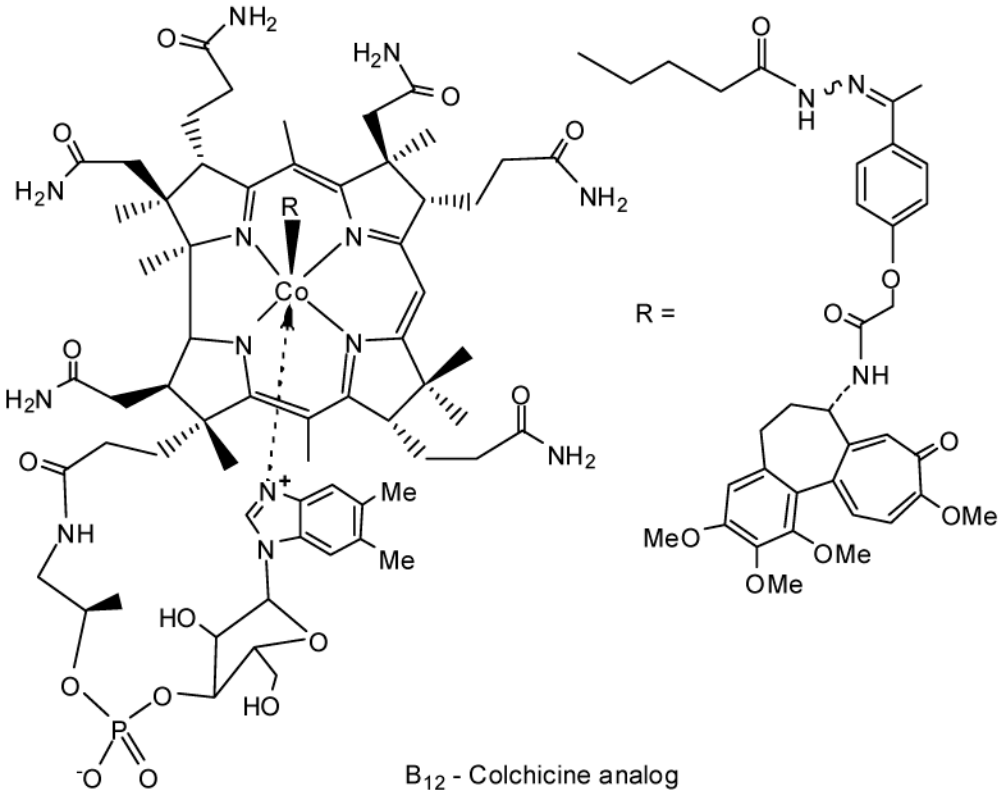


Fig. 3.

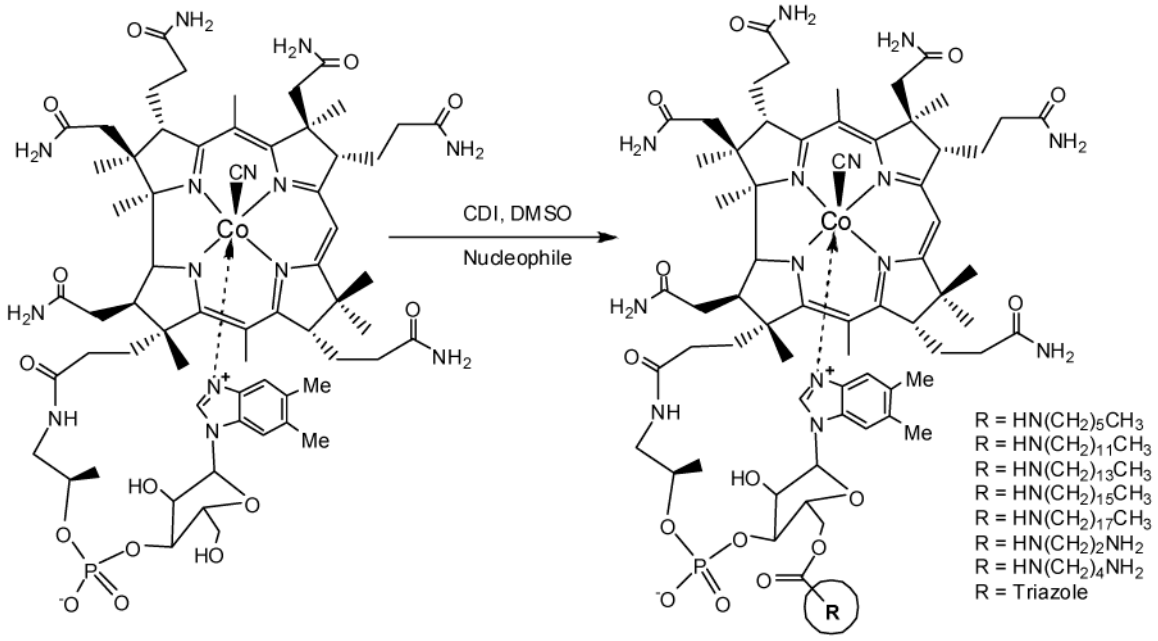


Fig. 4.

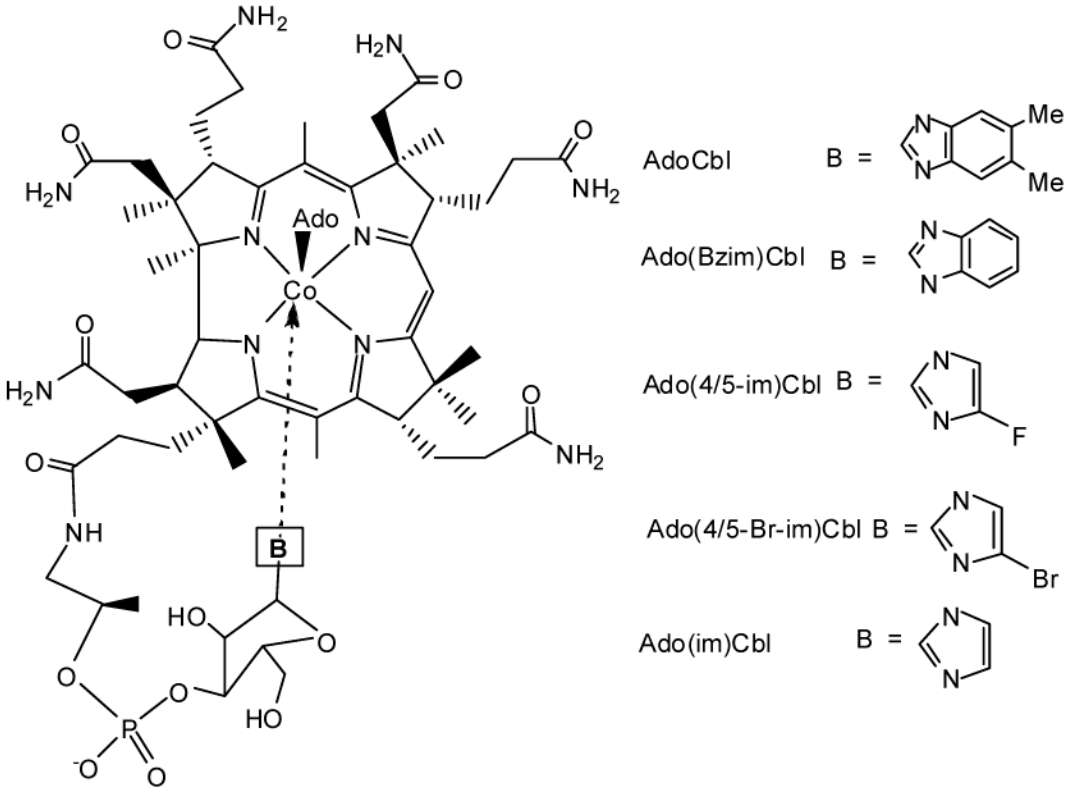


Fig. 5.
Analogs of co-enzyme B_{12}.

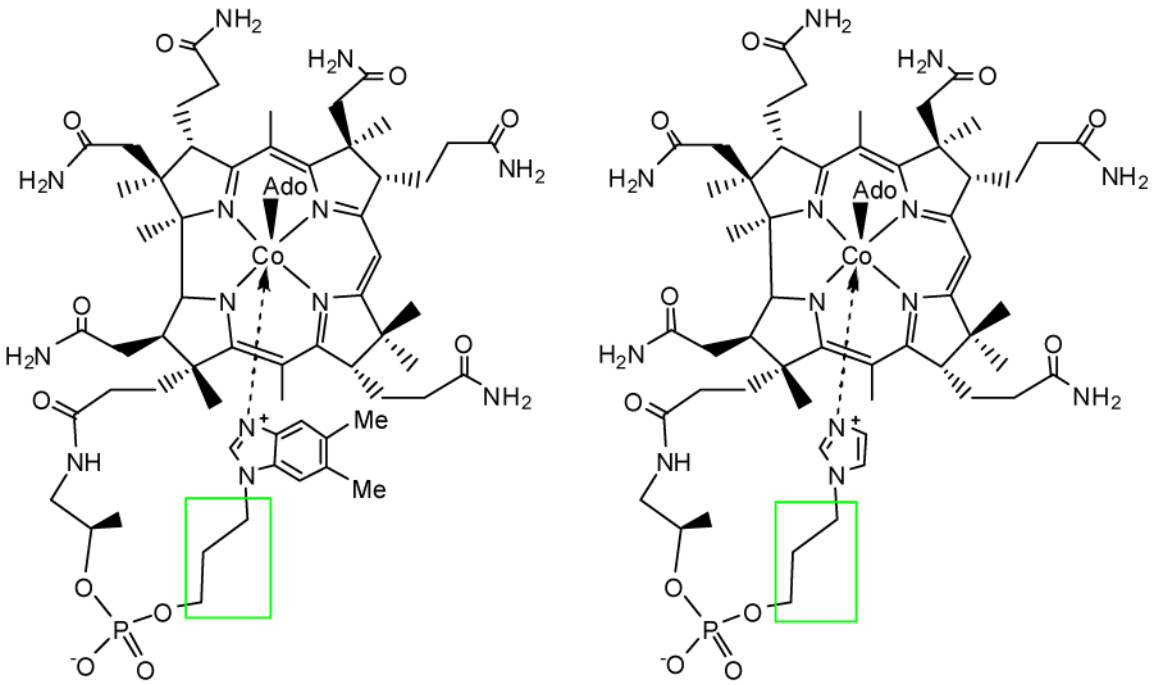


Fig. 6.

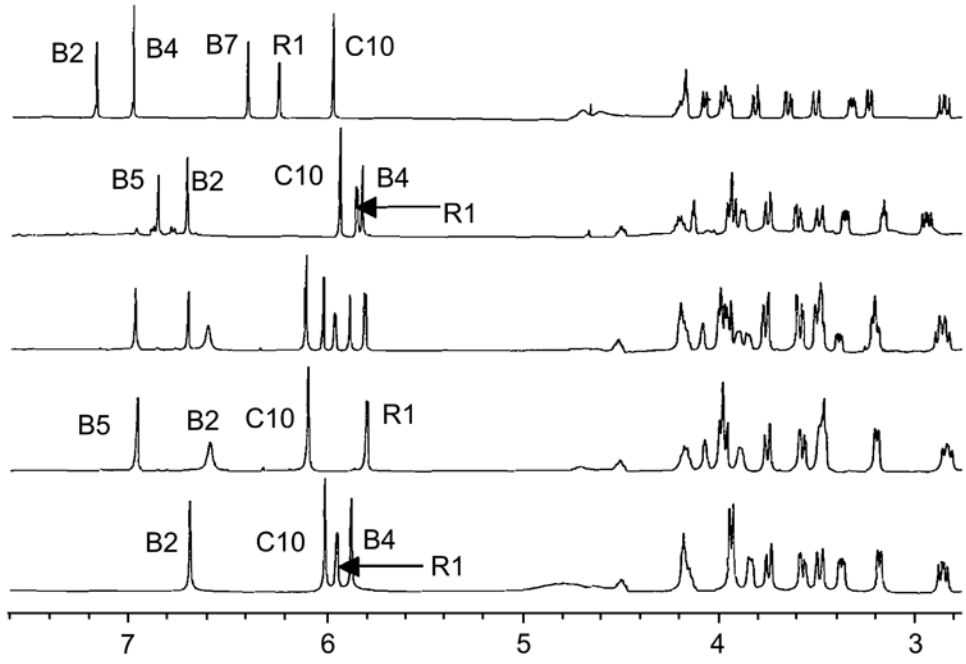


Fig 7.

From top lower field region ^{1}H NMR of B_{12}, CN(im)Cbl, mix of CN(4/5-im)Cbl, CN(4-im) Cbl, & CN(5-im)Cbl

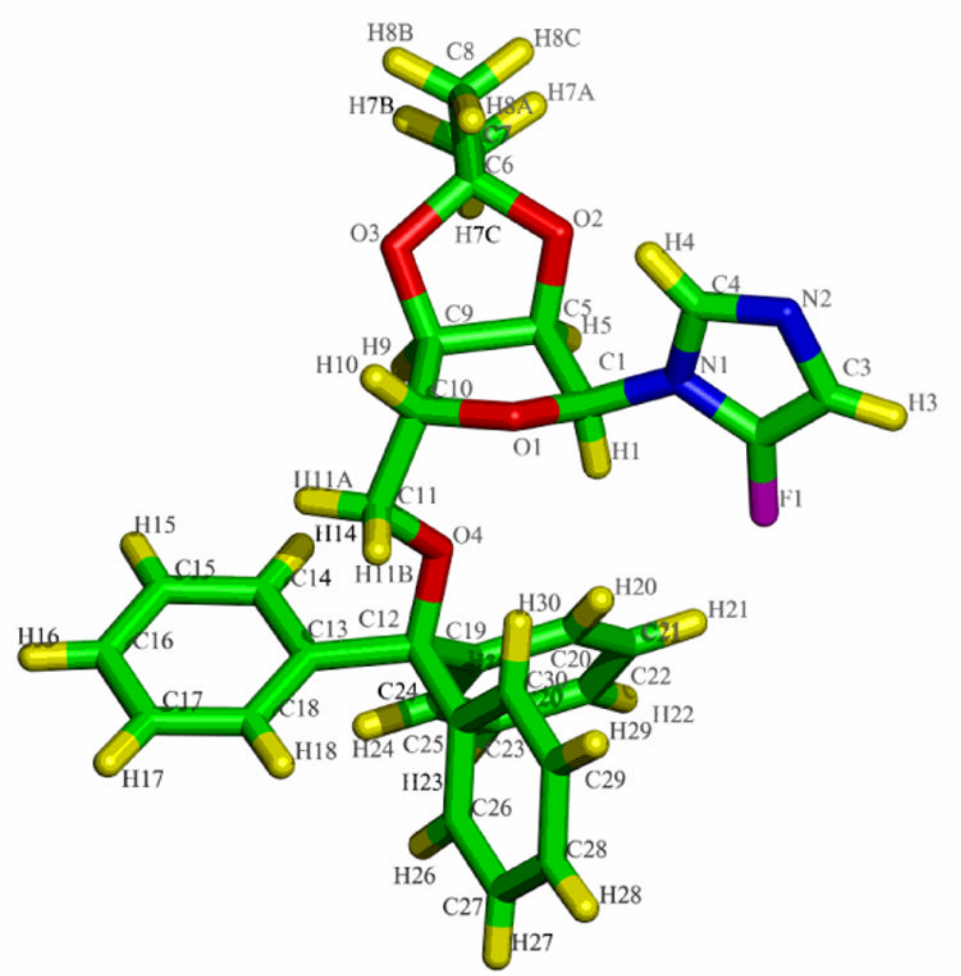


Fig 8.
X-ray structure of α-5-fluoroimidazole nucleoside **35**

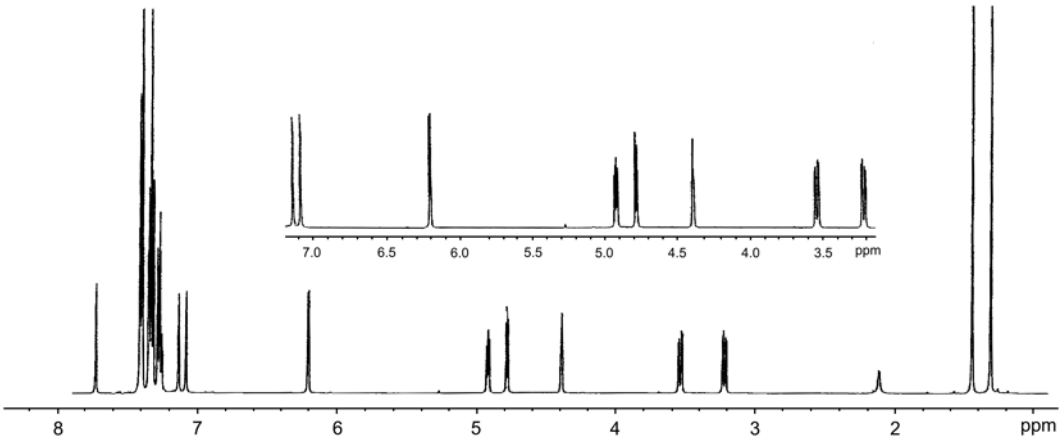


Fig 9.
^{1}H NMR of α-Imidazole ribonucleoside

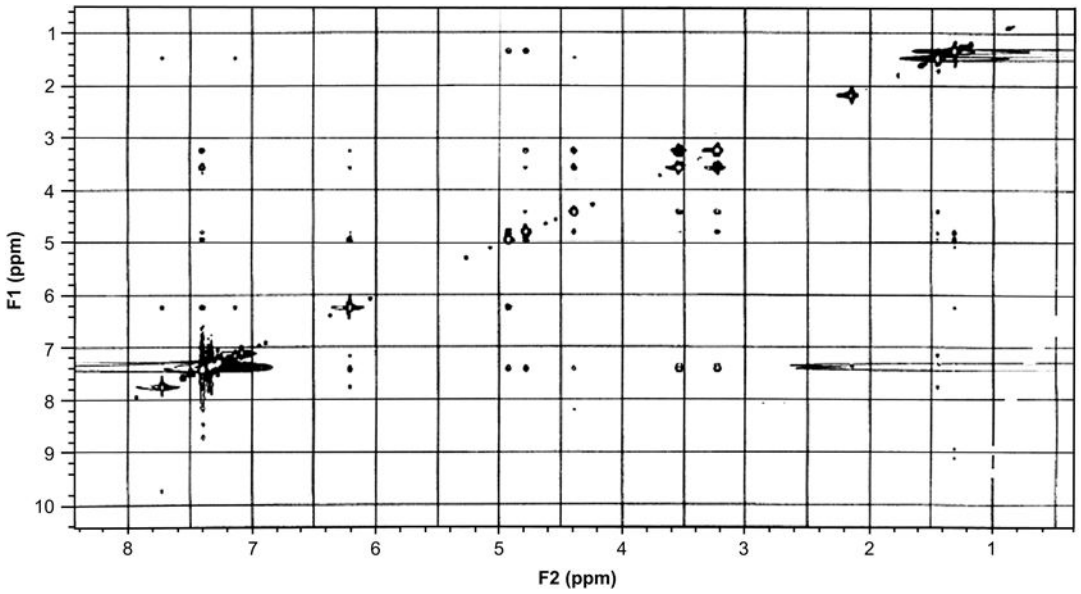


Fig 10.
nOe of 1-(5′-*O*-triphenylmethyl-2′,3′-*O*-isopropylidene-α-D-ribofuranosyl)-imidazole

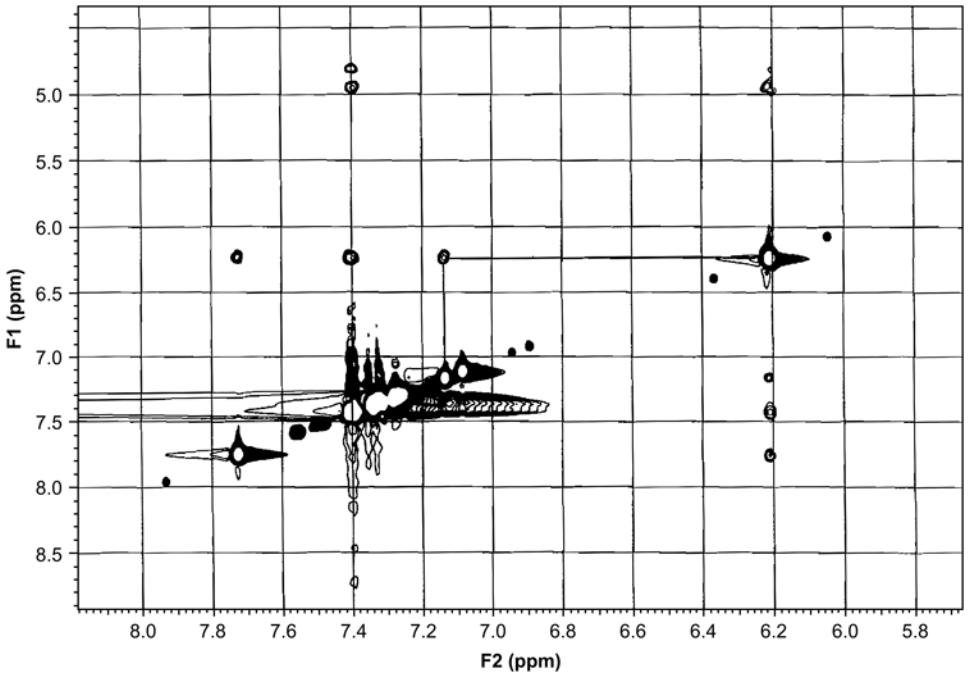


Fig 11.
Down field region nOe of α-imidazole ribonucleoside showing nOe between anomeric proton and C-5 imidazole proton

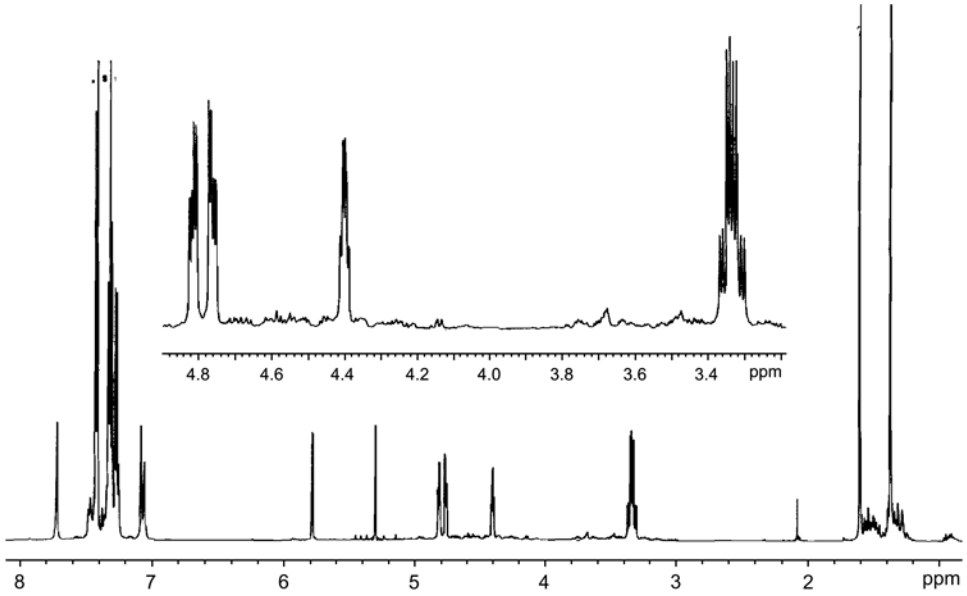


Fig 12.
^{1}H NMR of β-Imidazole ribonucleoside

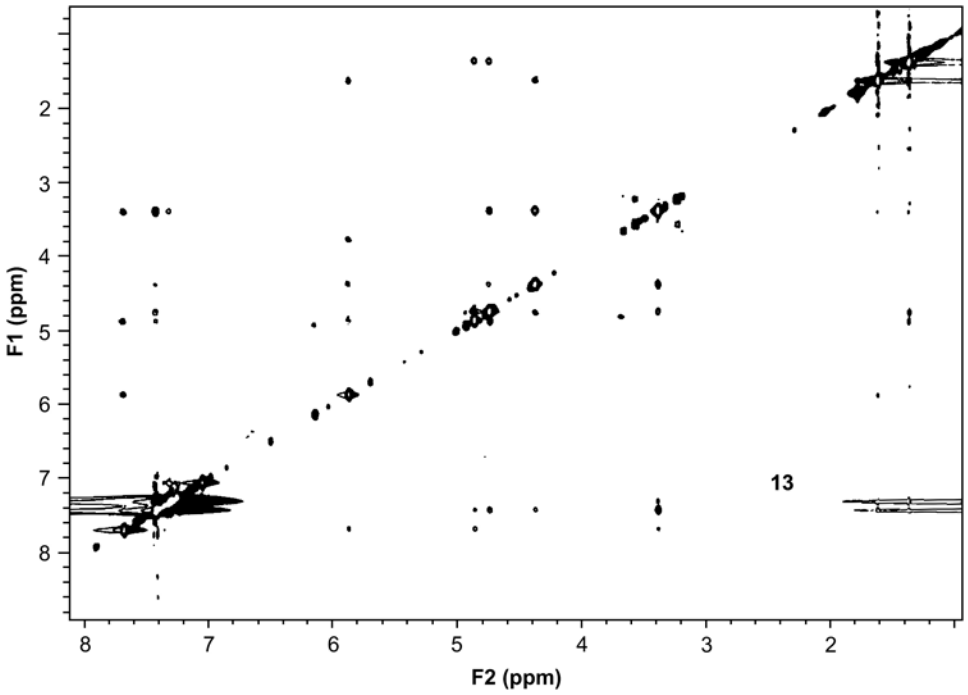


Fig 13.
nOe of 1-(5′-O-triphenylmethyl-2′,3′-O-isopropylidene-β-D-ribofuranosyl)-5-bromoimidazole

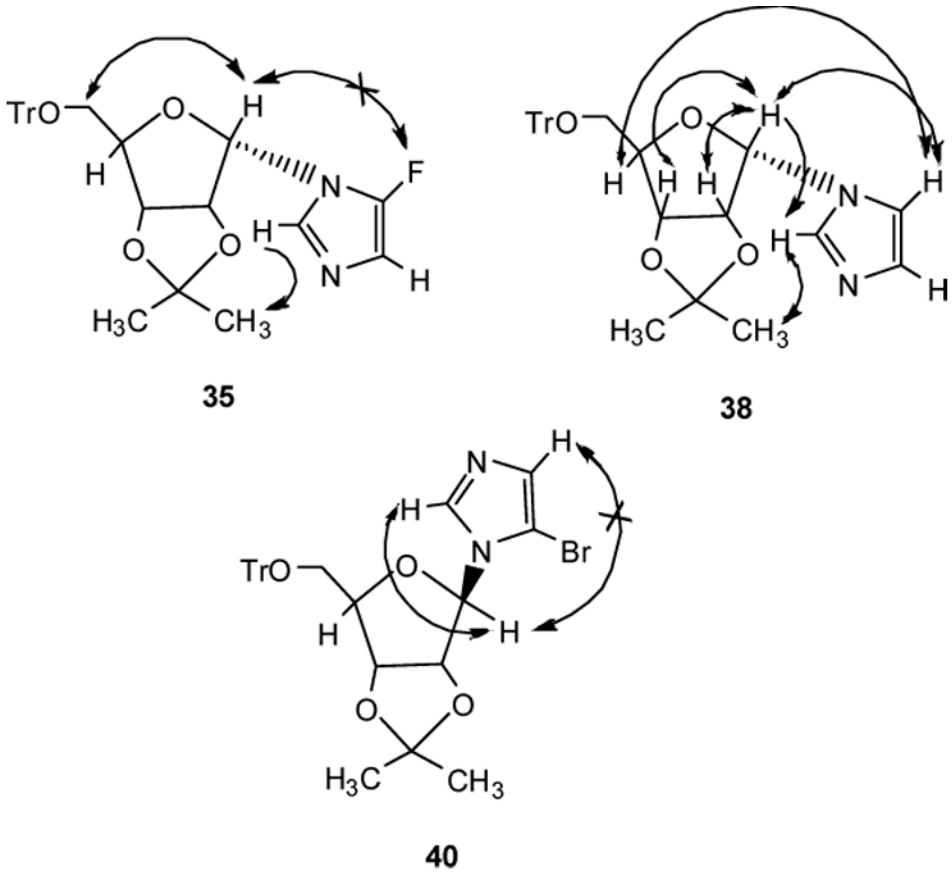


Fig 14.
nOe's of α/*β* 5-haloimidazole ribonucleosides

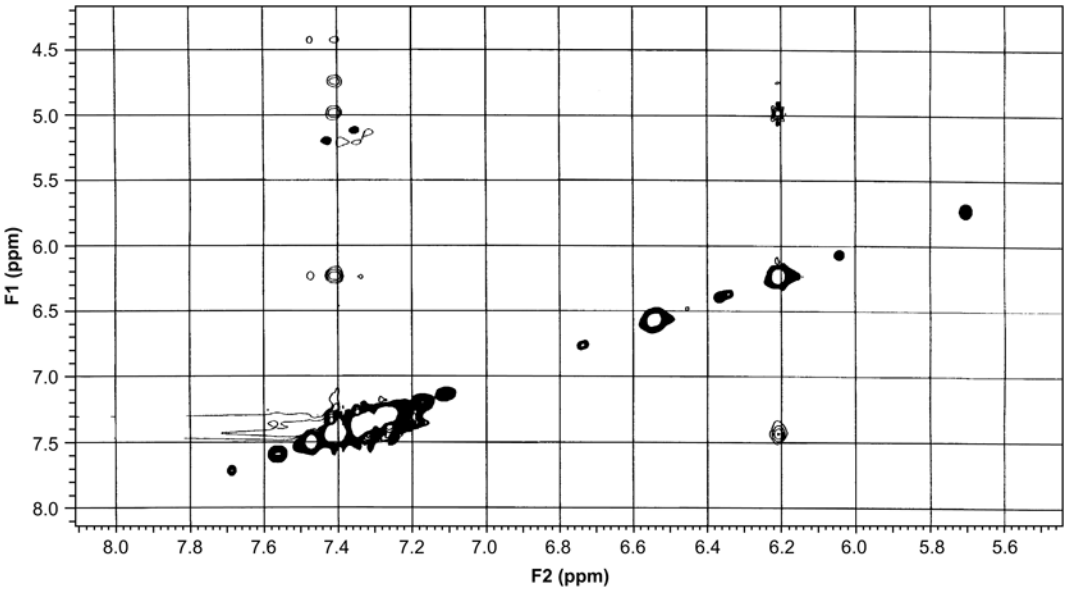


Fig 15.
Down field region nOe of 1-(5′-O-triphenylmethyl-2′,3′-O-isopropylidene-α-D-ribofuranosyl)-5-fluoroimidazole

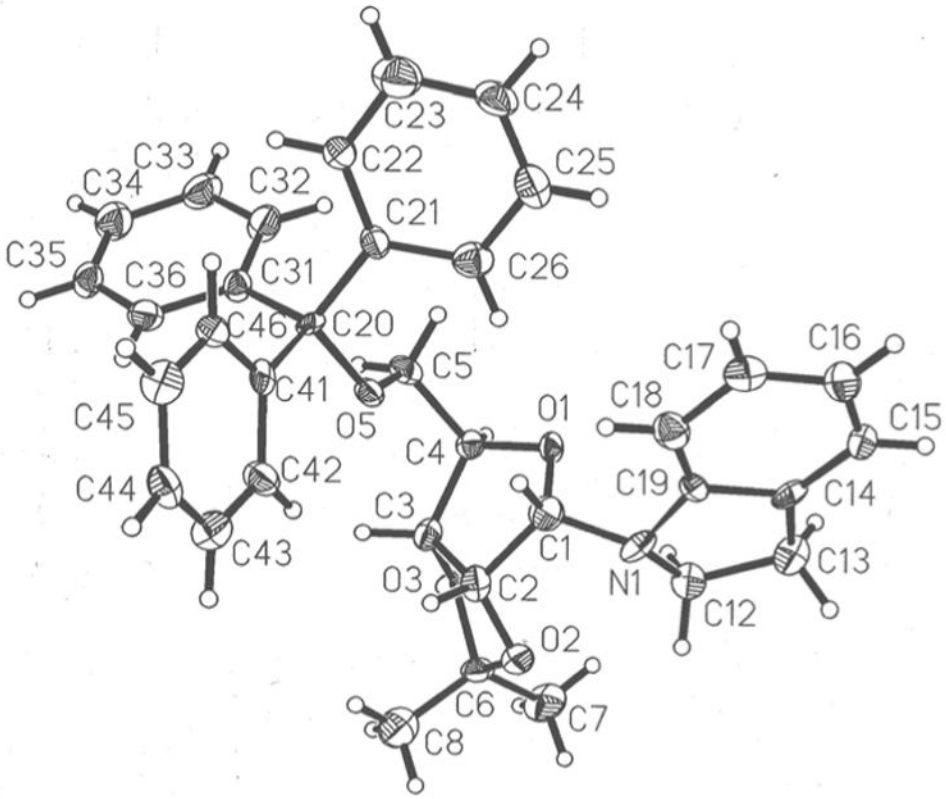


Fig 16.
ORTEP diagram of compound α-indoline ribonucleoside **79**

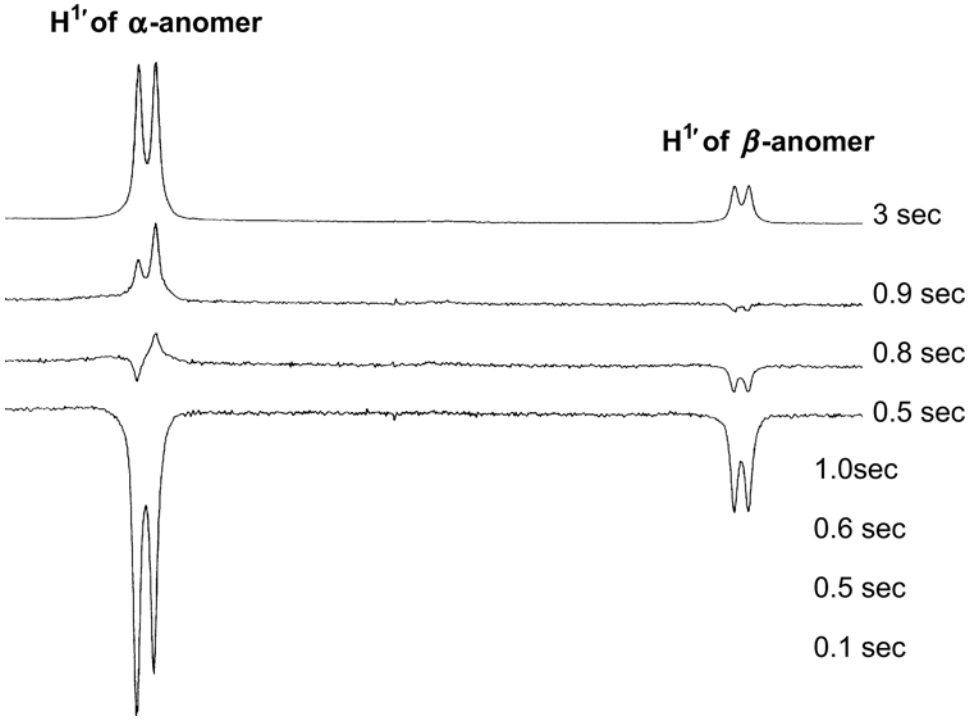
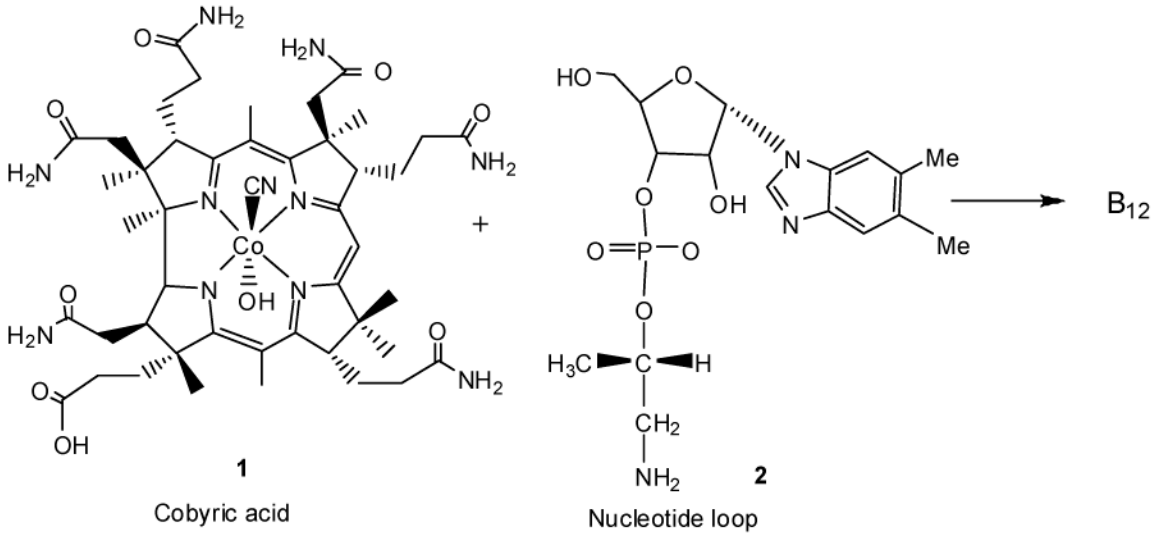
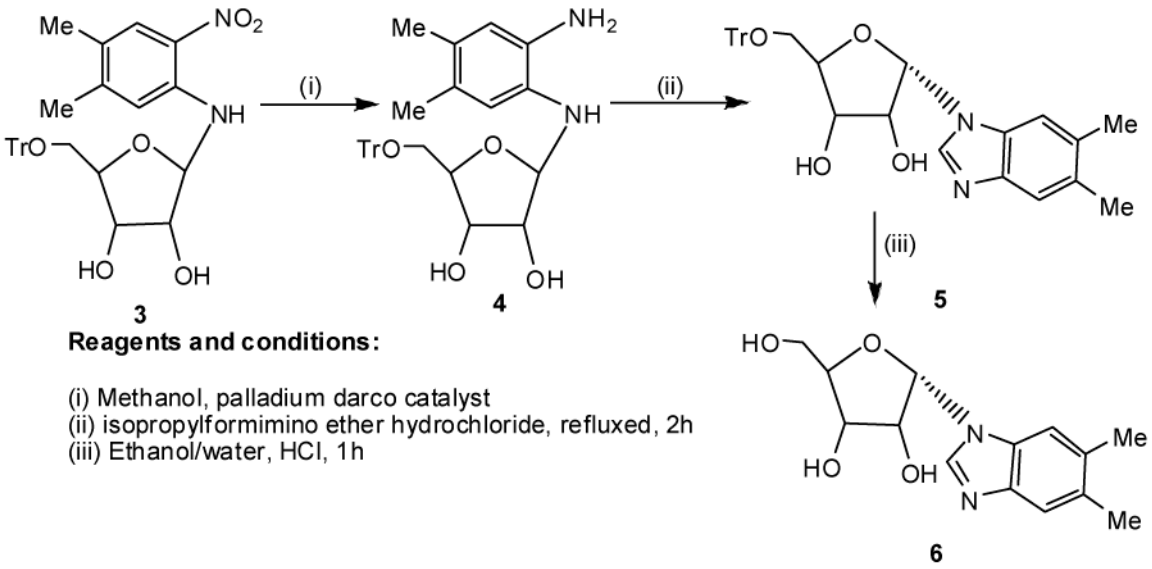


Figure 17.

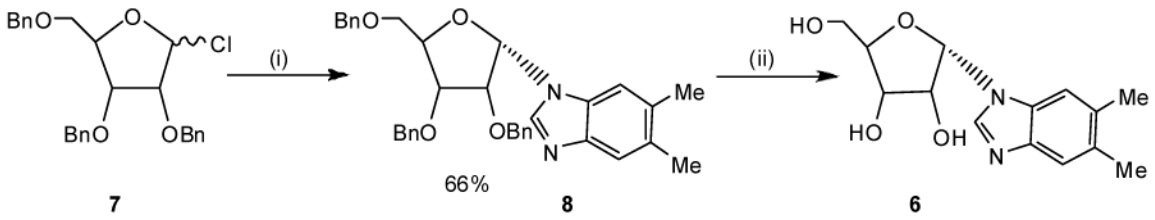
^{1}H inversion-recovery spectra of the mixture of **6** and **6b** (80% and 20%) for different mixing times. The $H^{1'}$ differential recovery for the α-anomer indicates the presence of dipole-csa cross correlations whereas the -*β* anomeric proton doublet shows no such effect. $H^{1'}$ chemical shift and $J_{H1'–H2'}$ 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 deutreated CD_3OD.



Scheme 1.



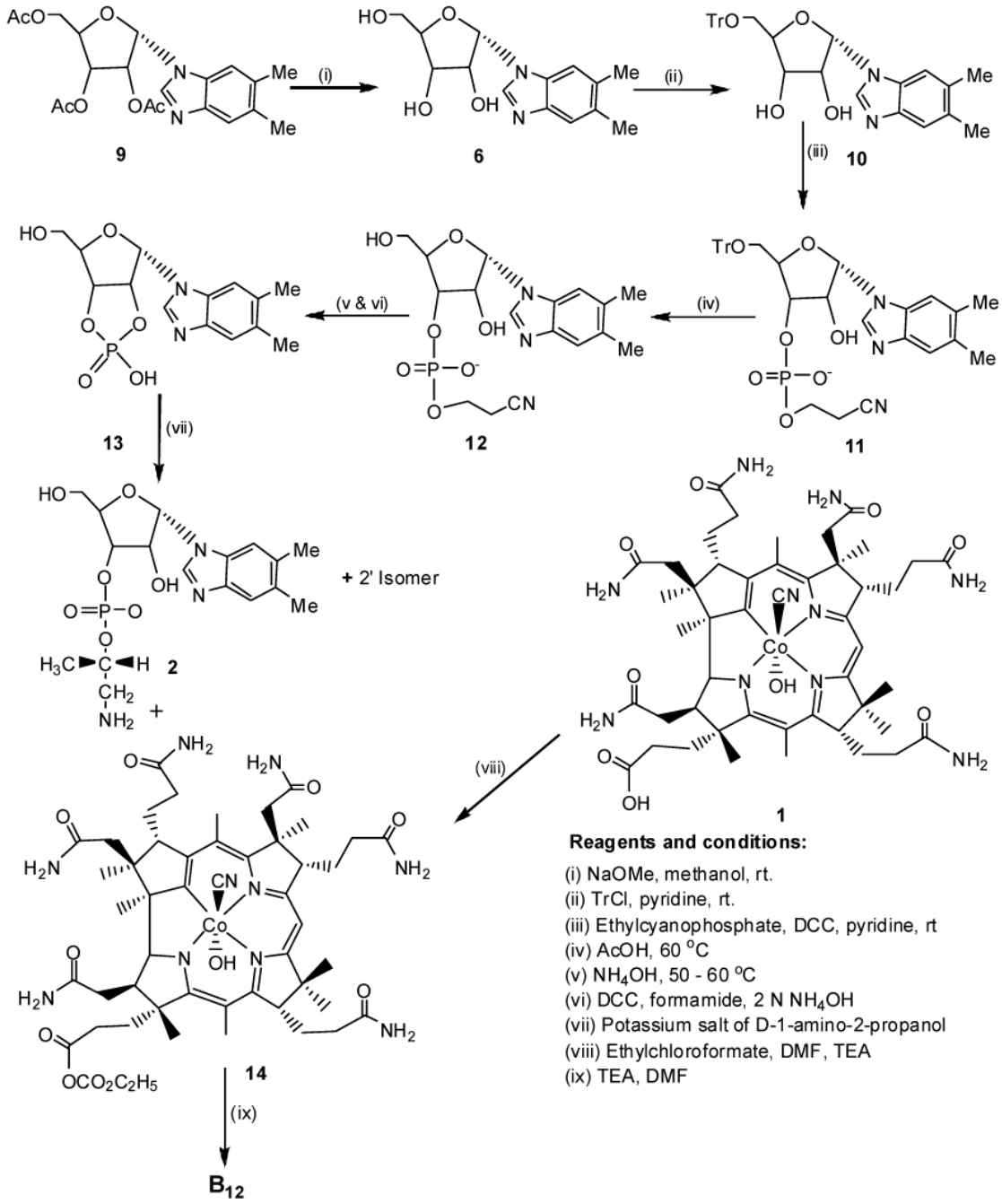
Scheme 2.



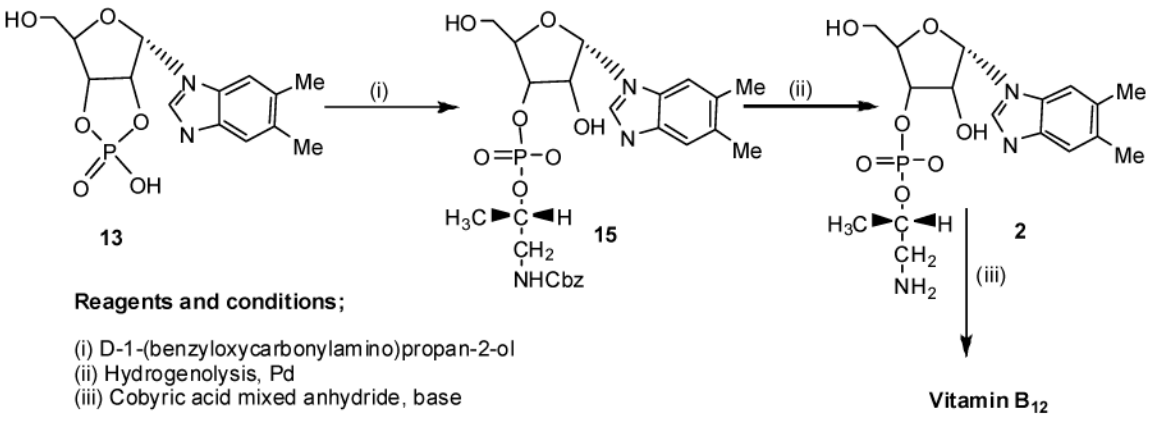
Reagents and conditions:

(i) Dimethylbenzimidazole, dioxane, 100 °C, 1.5, 66%
(ii) Methanol, palladium on charcoal, 94%

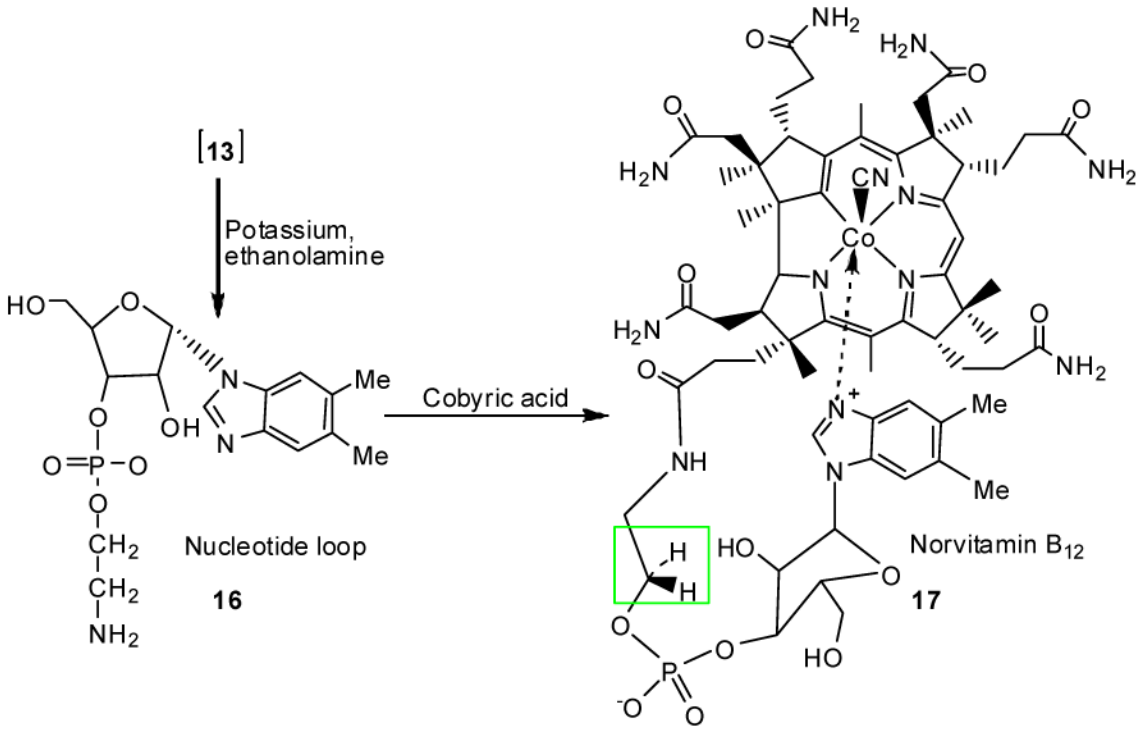
Scheme 3.



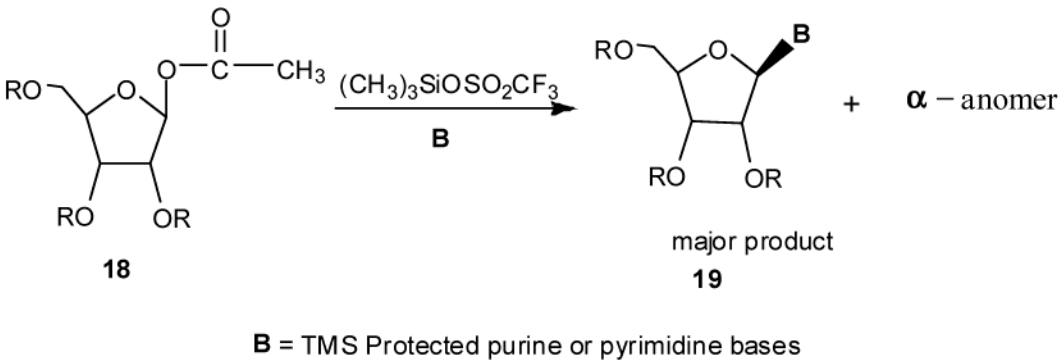
Scheme 4.



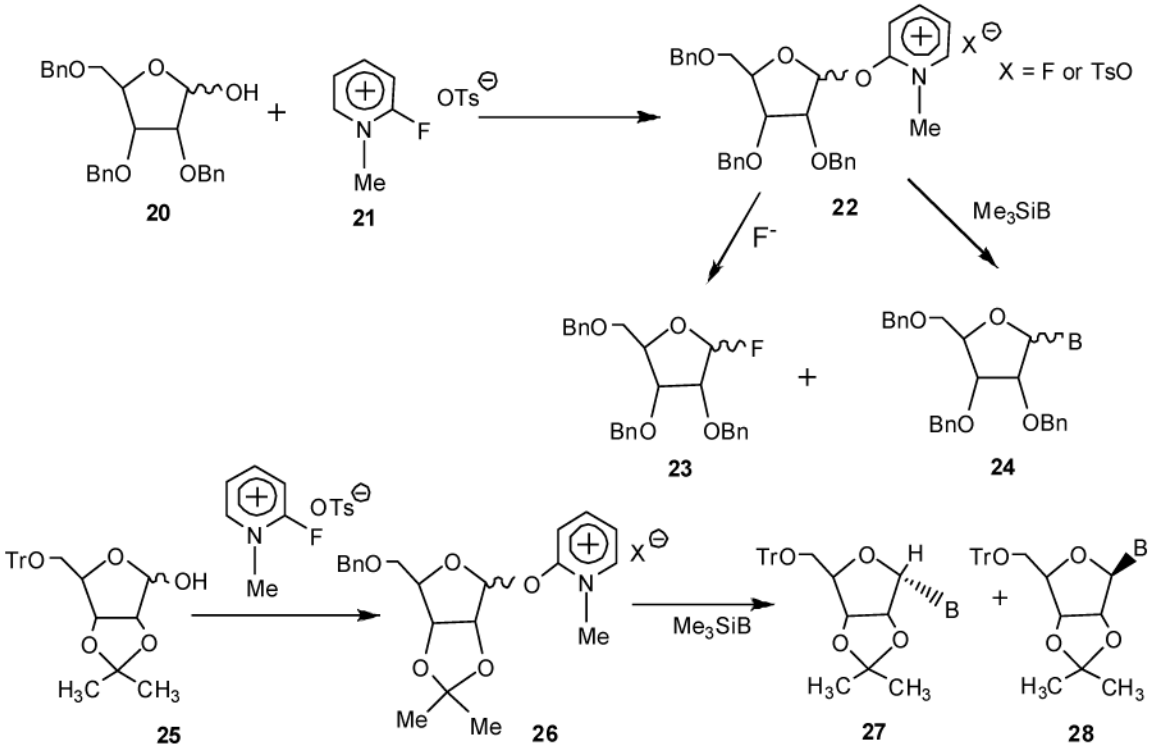
Scheme 5.



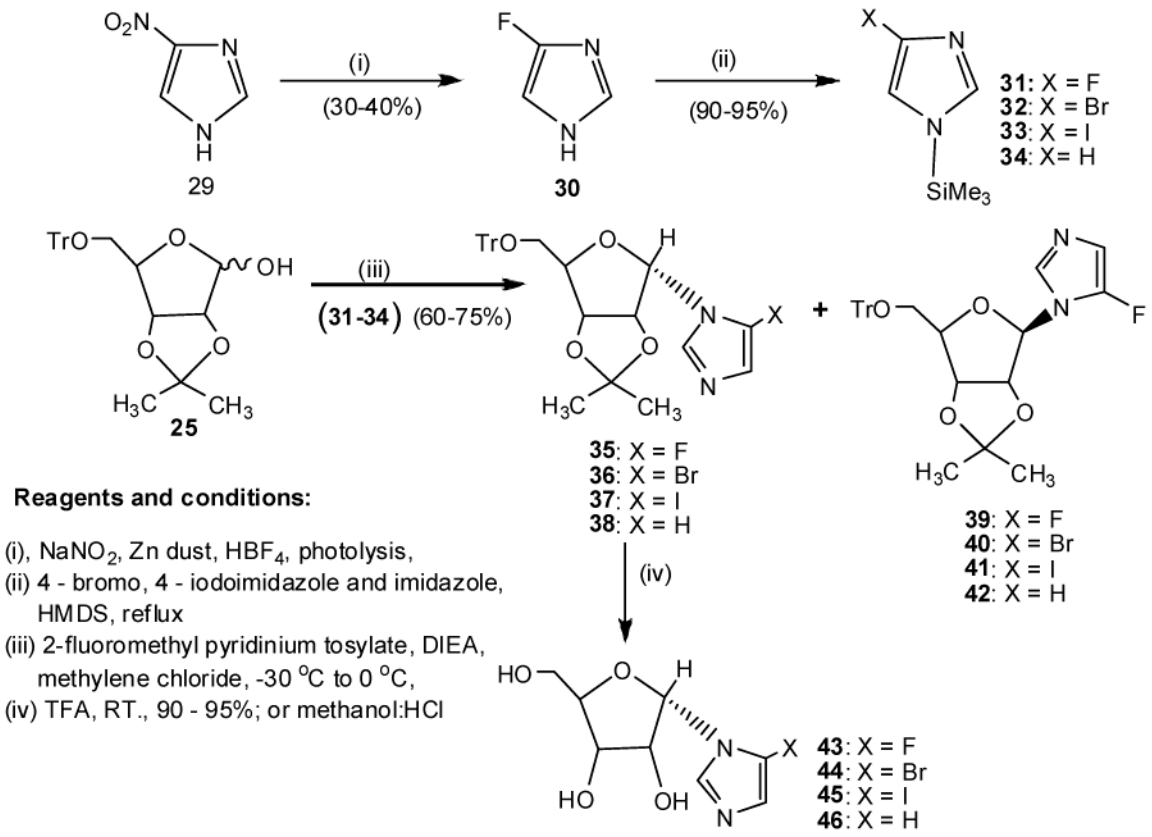
Scheme 6.



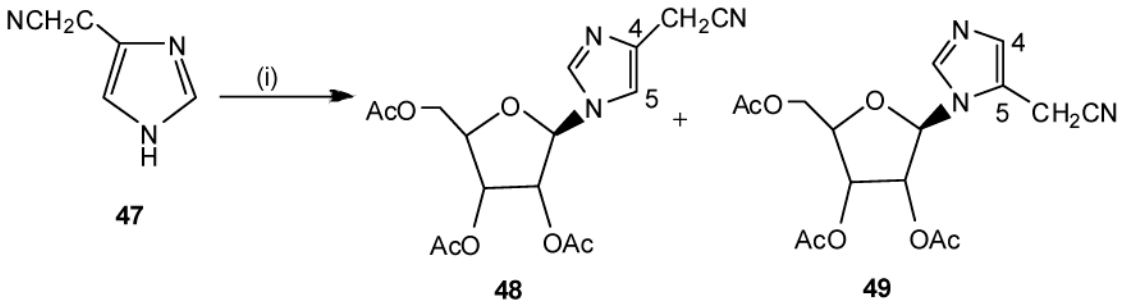
Scheme 7.



Scheme 8.



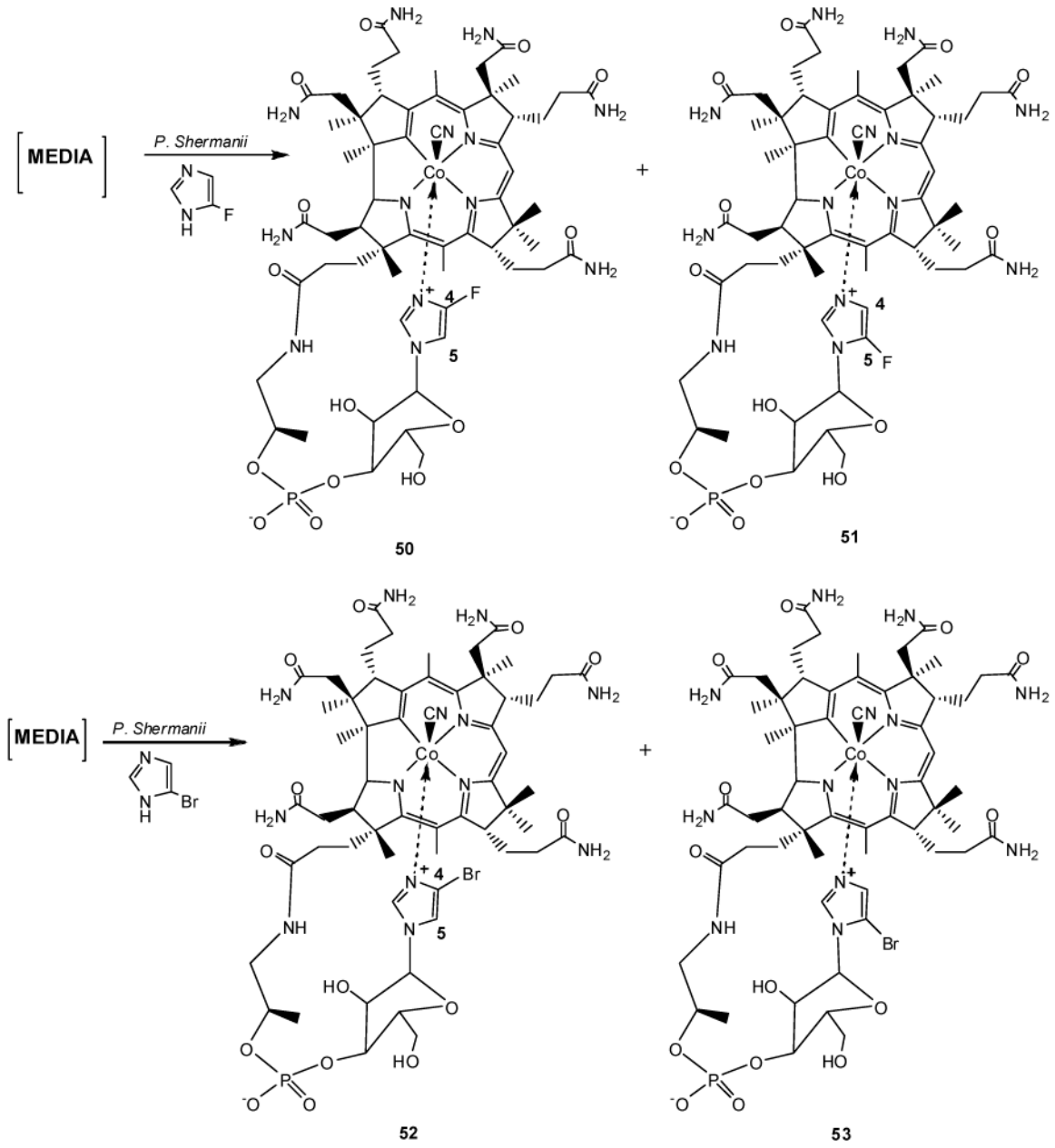
Scheme 9.



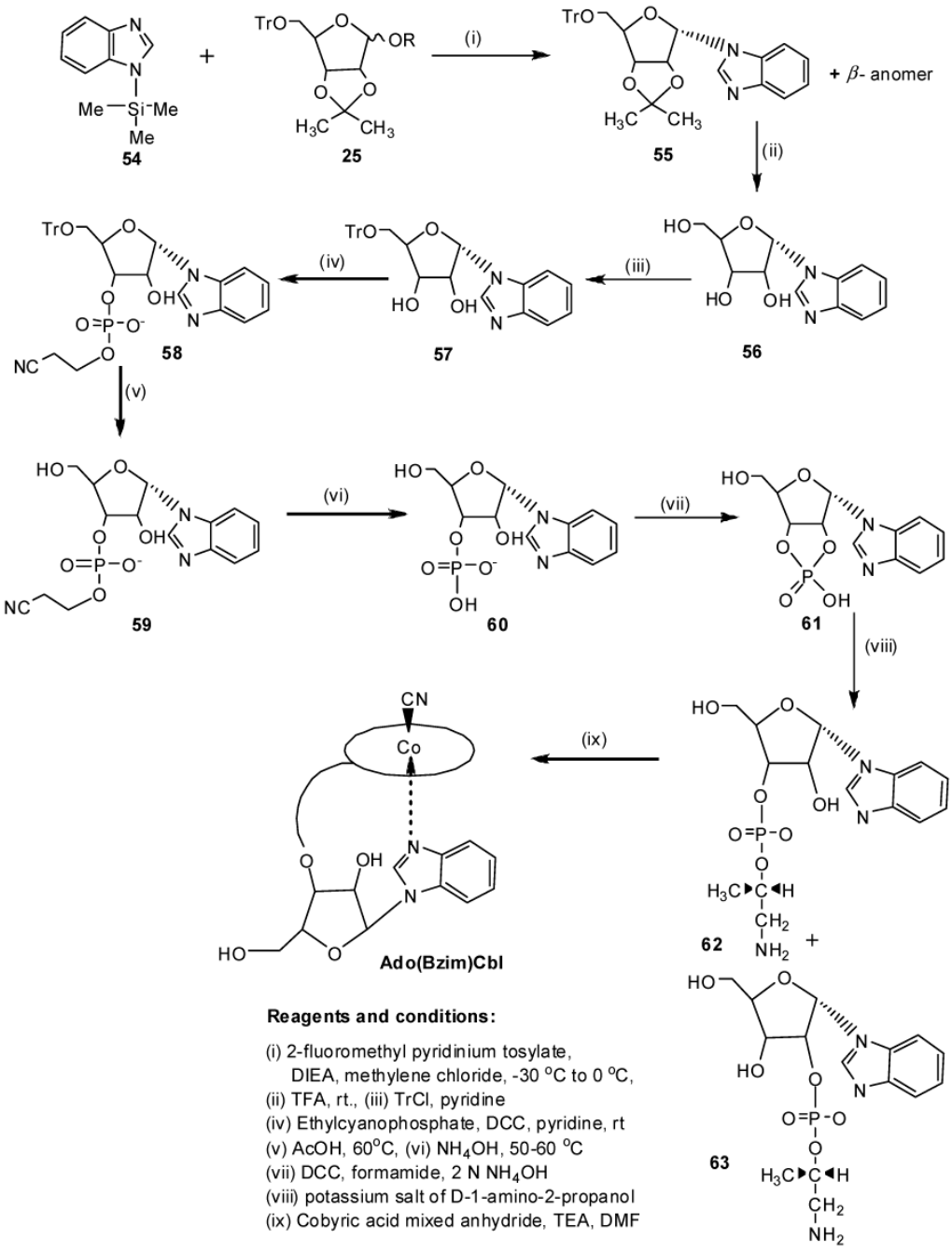
Reagents and conditions:

(i), BSA, TMSOTf, 60 °C, protected ribose

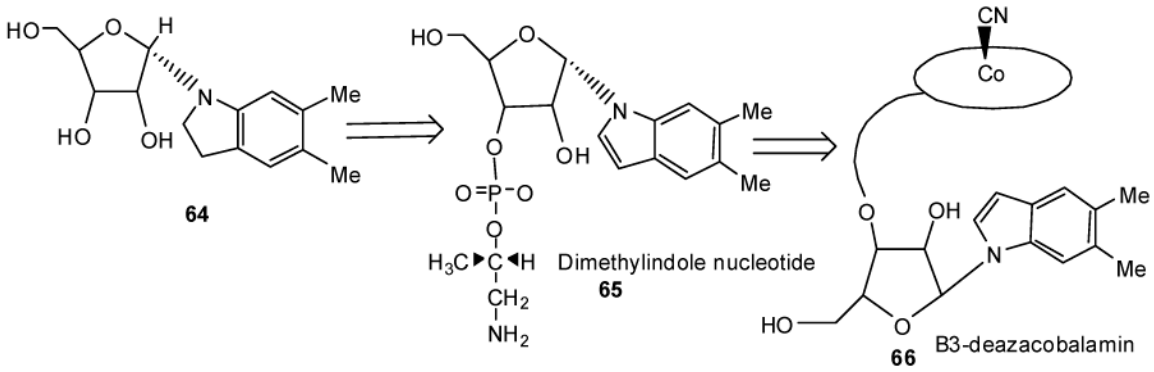
Scheme 10.



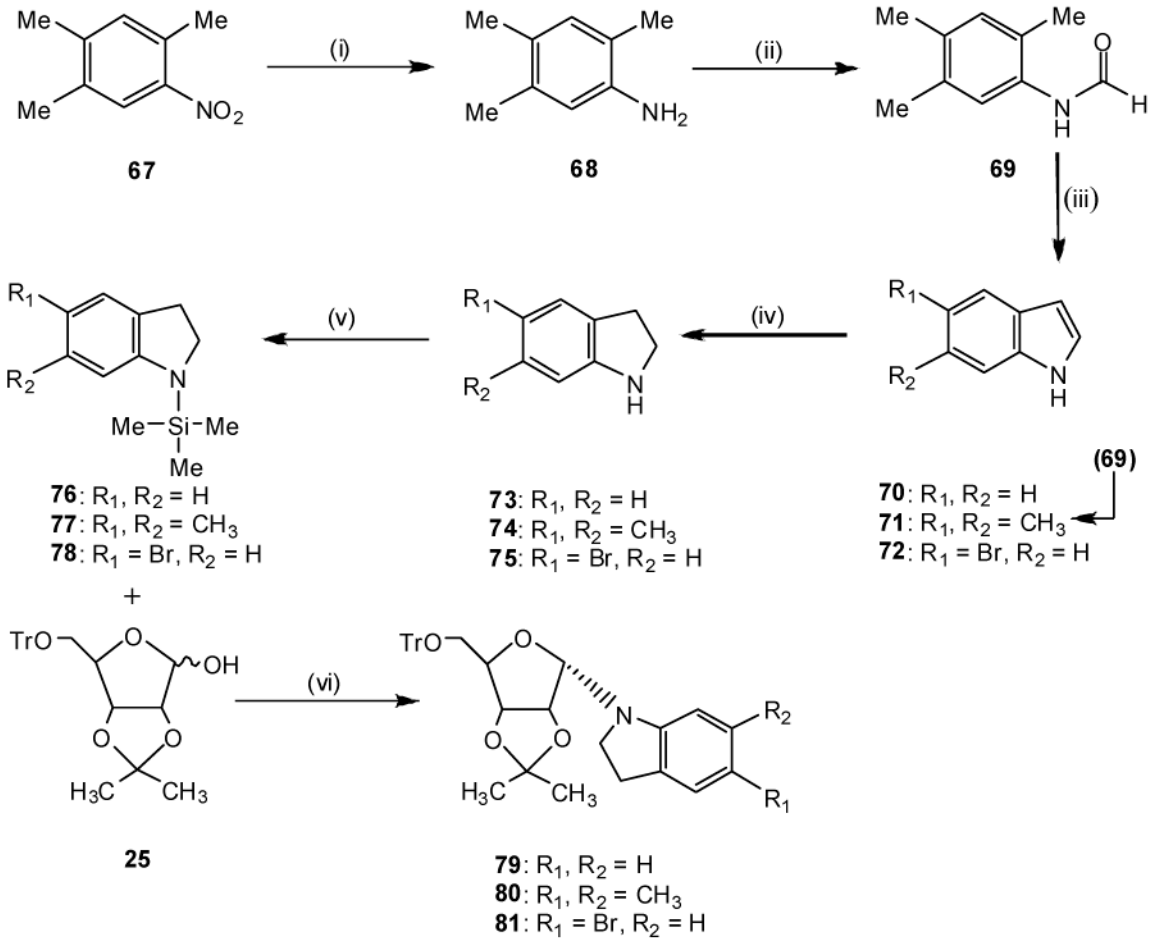
Scheme 11.



Scheme 12.



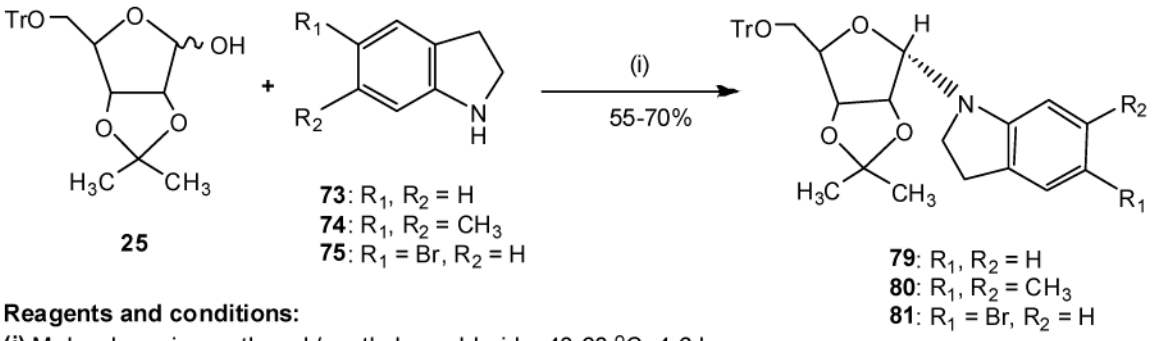
Scheme 13.



Reagents and conditions:

(i) Stannus chloride, HCl, reflux, 8 hr
(ii) Formic acid (80%), reflux
(iii) Potassium metal, *tert*-butanol, reflux / thermal cyclization 270-280 °C
(iv) Sodiumcyanoborohydride / AcOH
(v) Butyllithium / trimethylsilylchloride, -70 °C
(vi) 2-Fluoromethylpyridinium tosylate, *N,N′*-diisopropylethylamine, methylene chloride, 0 °C

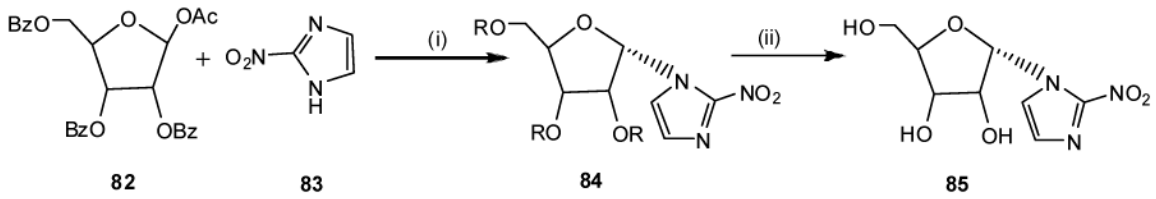
Scheme 14.



Reagents and conditions:

(i) Molecular seives, ethanol / methylene chloride, 40-60 °C, 4-6 h

Scheme 15.

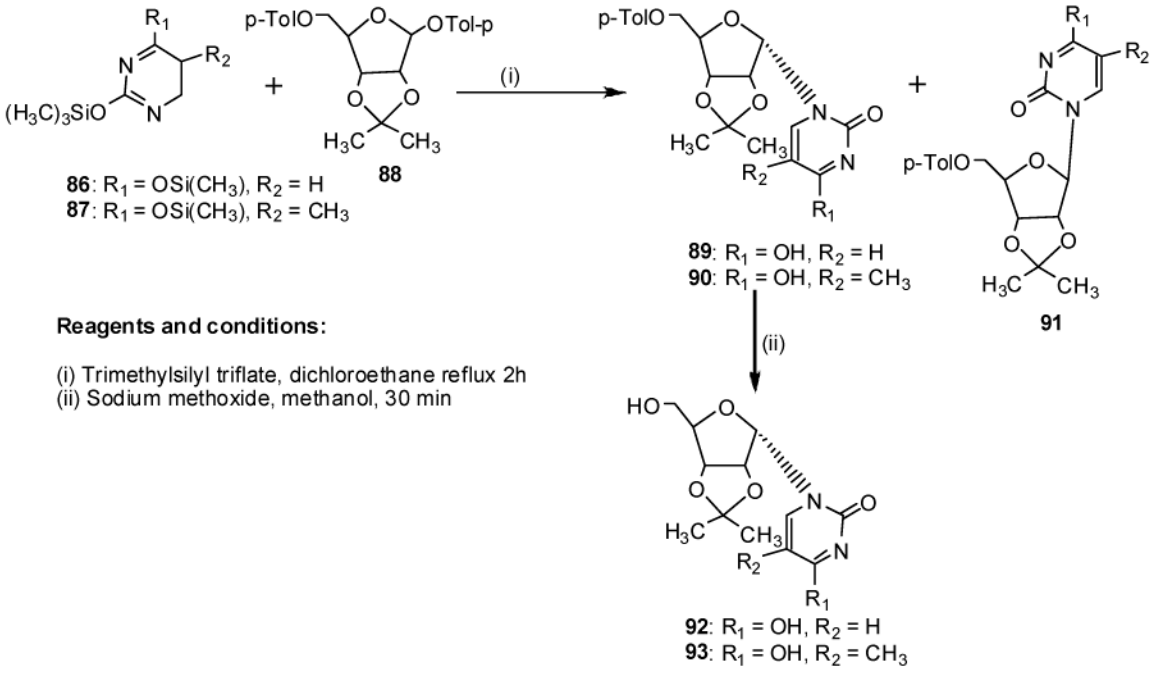


82 83 84 85

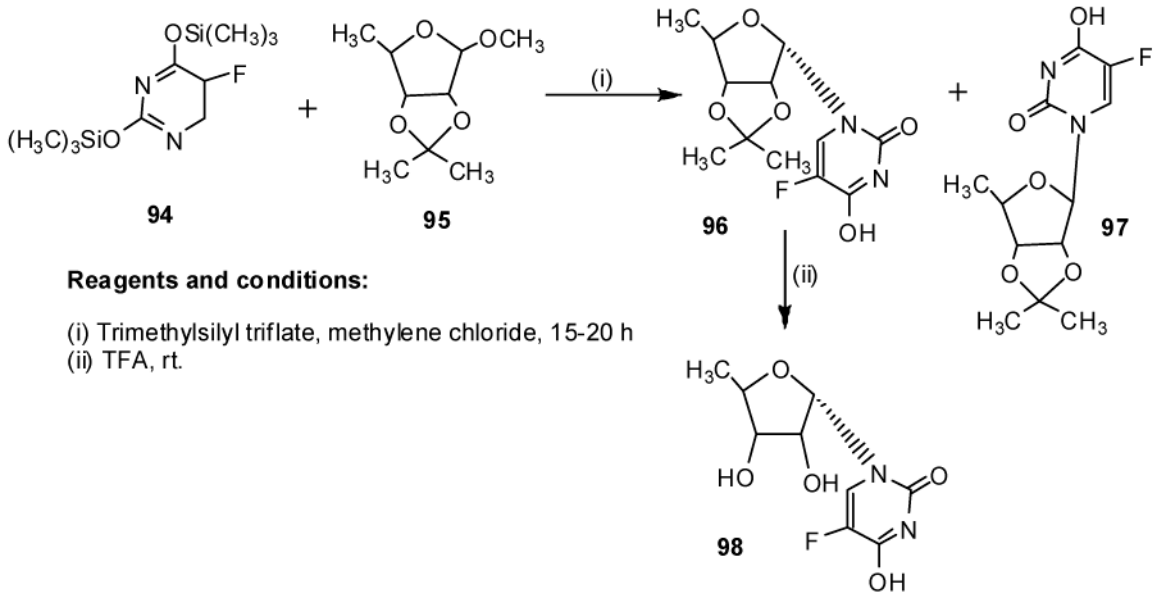
Reagents and conditions:

(i) Stannic chloride/mercuric cyanide, 61%
(ii) Hydrolysis

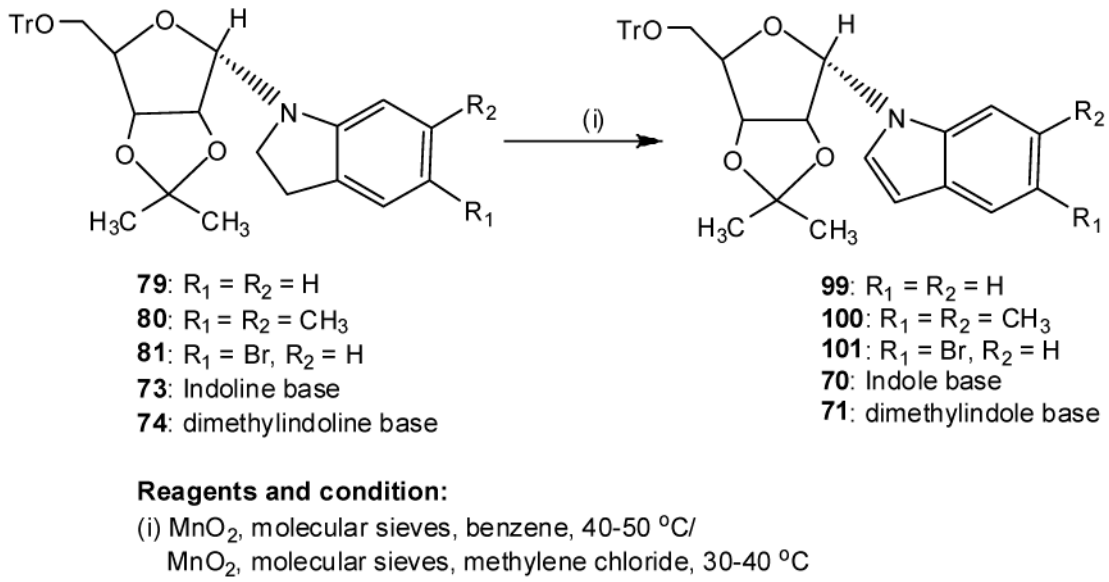
Scheme 16.



Scheme 17.



Scheme 18.



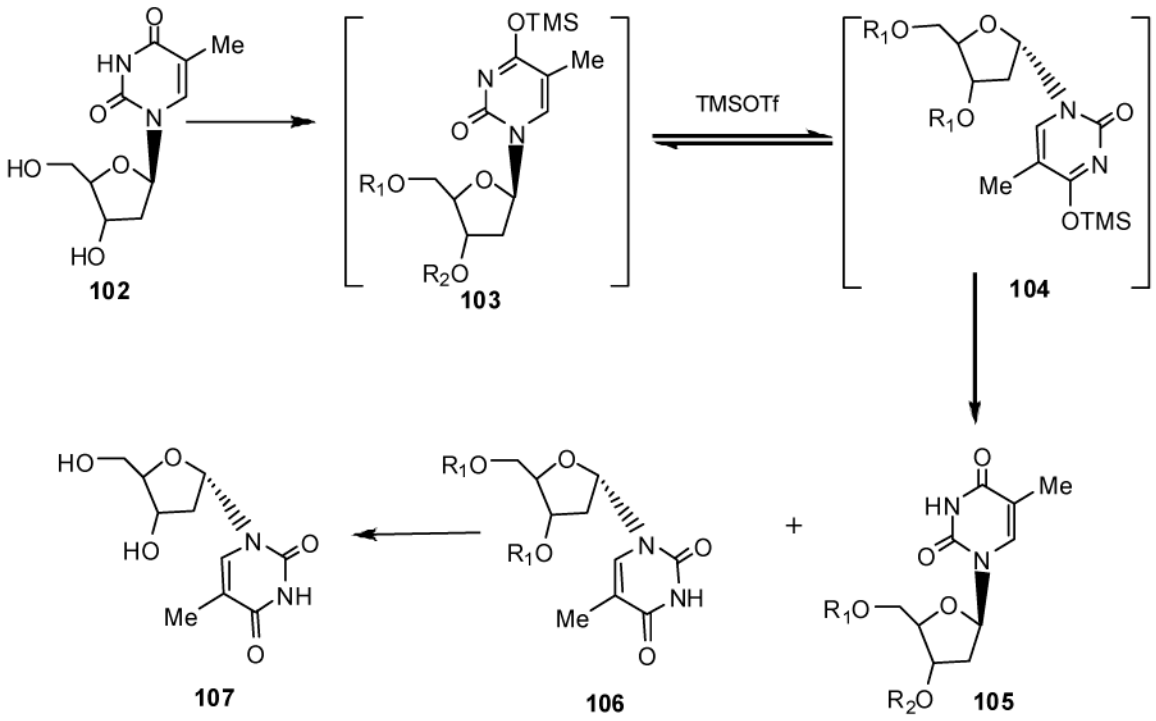
79: $R_1 = R_2 = H$
80: $R_1 = R_2 = CH_3$
81: $R_1 = Br$, $R_2 = H$
73: Indoline base
74: dimethylindoline base

99: $R_1 = R_2 = H$
100: $R_1 = R_2 = CH_3$
101: $R_1 = Br$, $R_2 = H$
70: Indole base
71: dimethylindole base

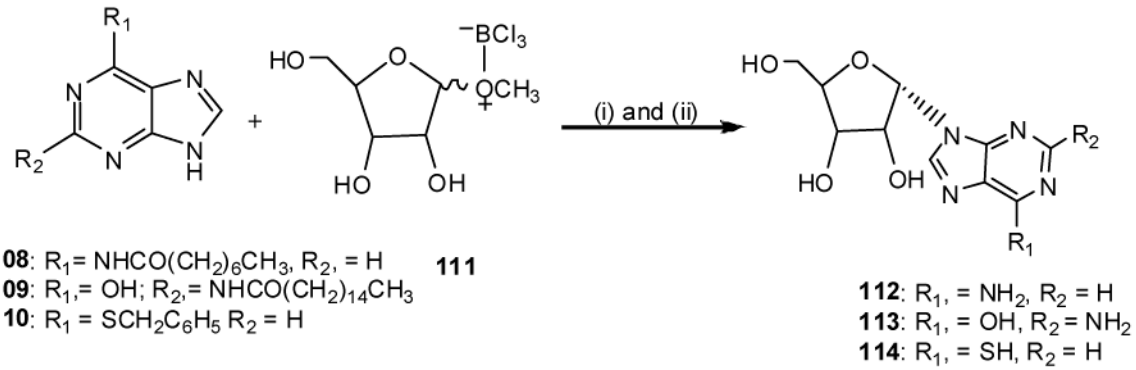
Reagents and condition:

(i) MnO_2, molecular sieves, benzene, 40-50 °C/
MnO_2, molecular sieves, methylene chloride, 30-40 °C

Scheme 19.



Scheme 20.



108: R_1= $NHCO(CH_2)_6CH_3$, R_2, = H
109: R_1,= OH; R_2,= $NHCO(CH_2)_{14}CH_3$
110: R_1 = $SCH_2C_6H_5$ R_2 = H

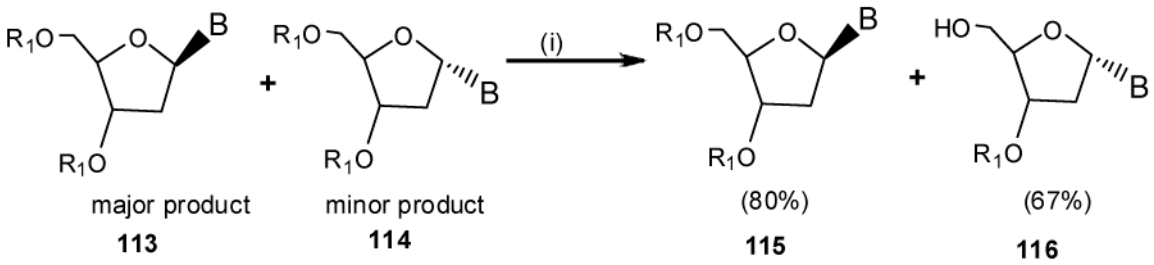
111

112: R_1, = NH_2, R_2 = H
113: R_1, = OH, R_2 = NH_2
114: R_1, = SH, R_2 = H

Reagents and conditions:

(i) Pyridine, chloroform, reflux, 3h
(ii) Sodium methoxide

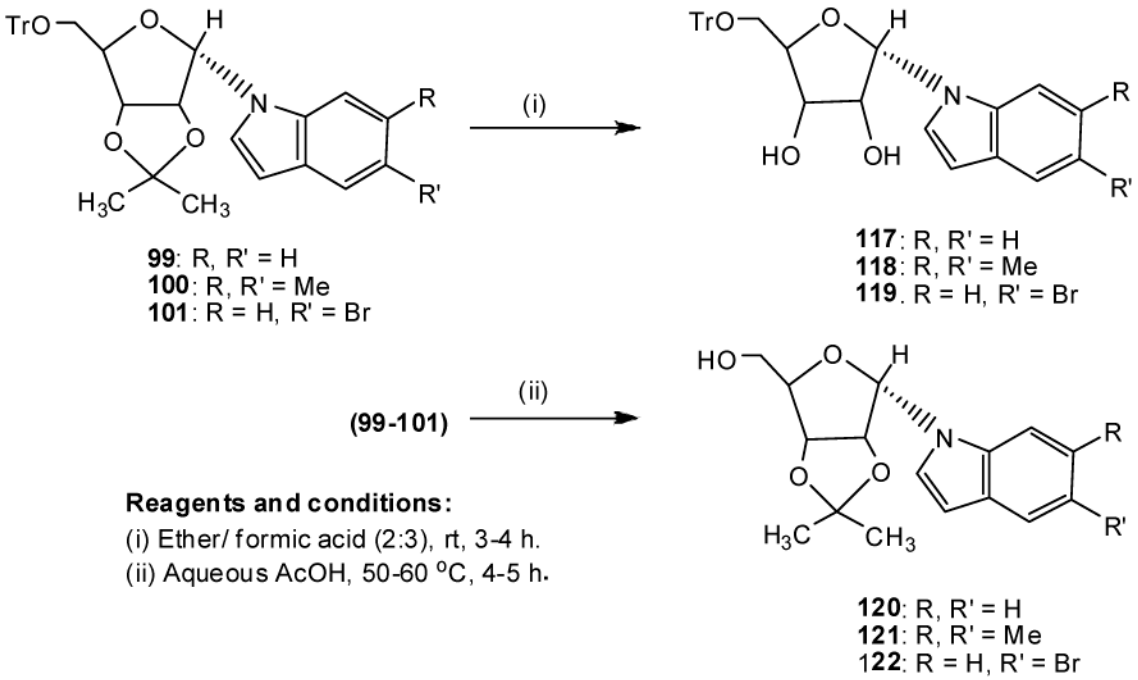
Scheme 21.



(Mixture of α and β thymidine 8:2 ratio)
R_1 = P-Cl-benzoyl
B = Thymine

Reagents and conditions:
(i) Lipase, dioxane, 0.15 M phosphate buffer, 164 h, 60 ^{0}C

Scheme 22.

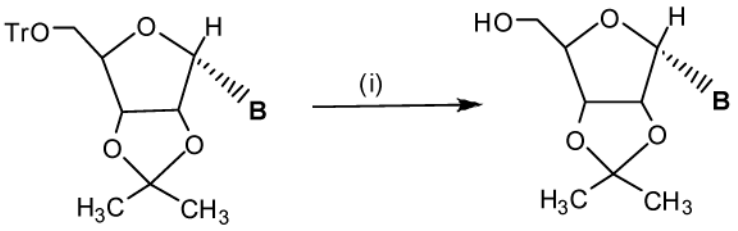


99: R, R' = H
100: R, R' = Me
101: R = H, R' = Br

117: R, R' = H
118: R, R' = Me
119. R = H, R' = Br

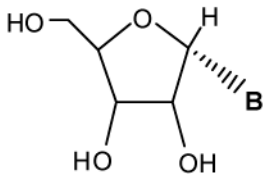
120: R, R' = H
121: R, R' = Me
122: R = H, R' = Br

Scheme 23.



55: B = benzimidazole
123: B = dimethylbenzimidazole
36: B = 5-bromoimidazole
38: B = imidazole

124: B = benzimidazole
125: B = dimethylbenzimidazole
126: B = 5-bromoimidazole
127: B = imidazole



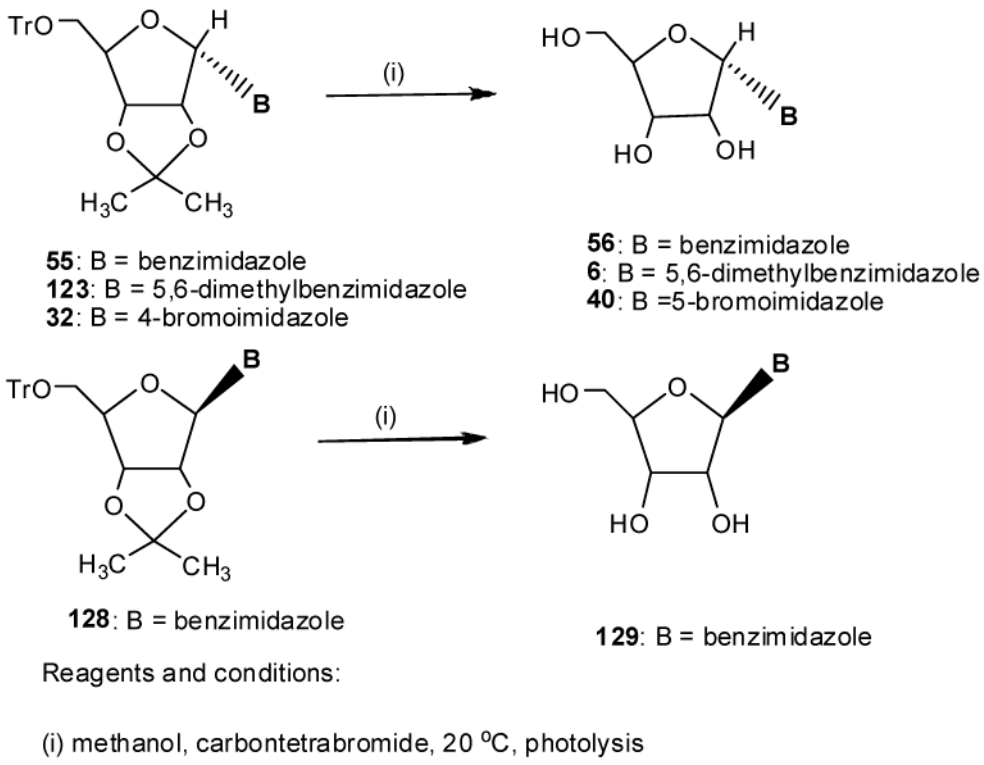
36, 38, 55, 123 —(ii)→

56: B = benzimidazole
6: B = dimethylbenzimidazole
44: B = 5-bromoimidazole
46: B = imidazole

Reagents and conditions:

(i) Ether/ formic acid, (2:3), room temp, 3-4 h, or 1% TFA, methylene chloride, room temp, 30 min or aqueous AcOH (80:20), 50-60 °C
(ii) 50% TFA: water

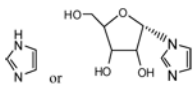
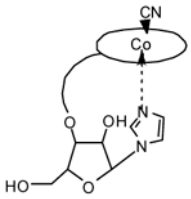
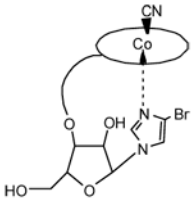
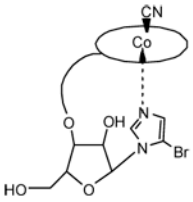
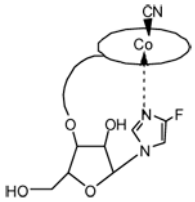
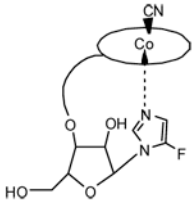
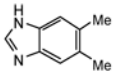
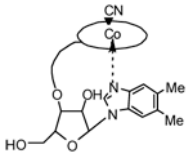
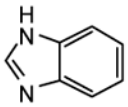
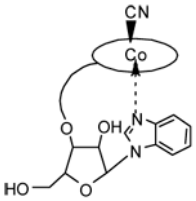
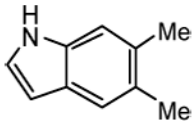
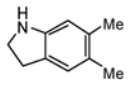
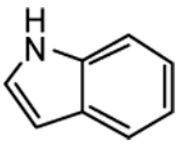
Scheme 24.



Scheme 25.

Table 1

Biosynthesis of analogs of AdoCbl using *Propionibacterium shermanii*[26]

Base added to the medium	Analogues		Ref. no.
			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

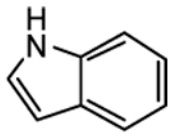
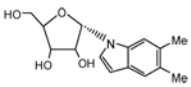
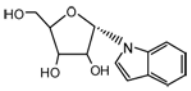
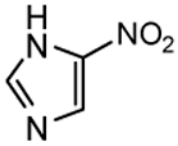
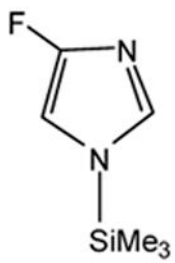
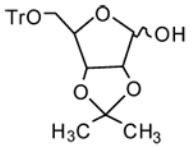
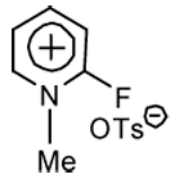
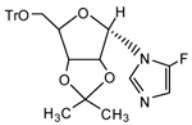
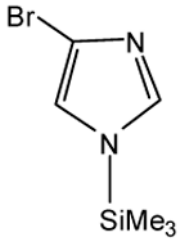
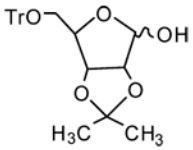
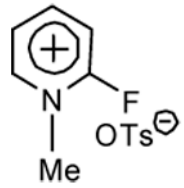
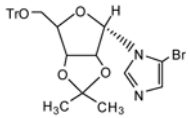
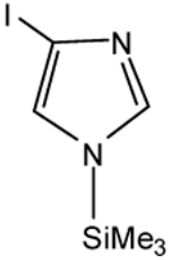
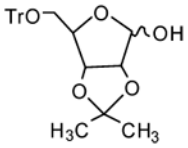
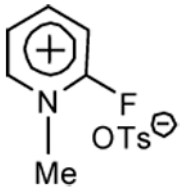
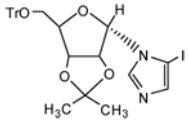
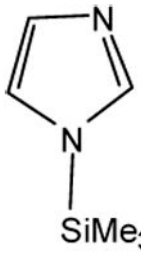
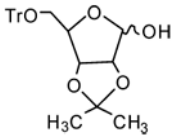
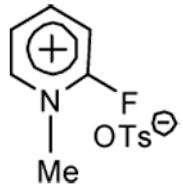
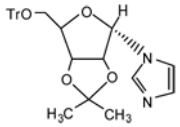
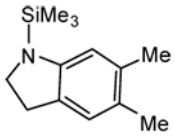
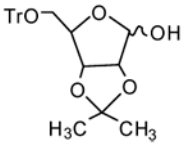
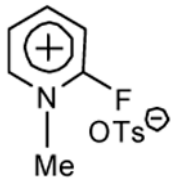
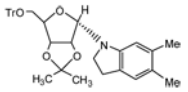
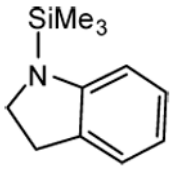
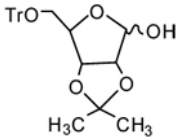
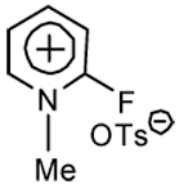
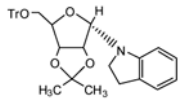
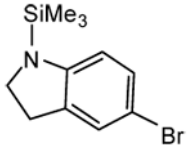
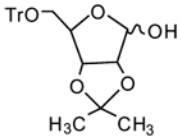
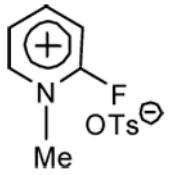
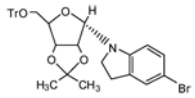
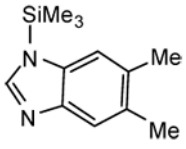
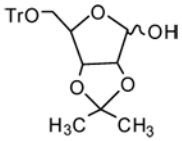
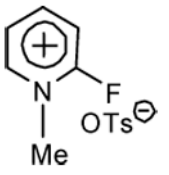
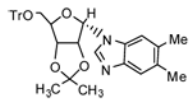
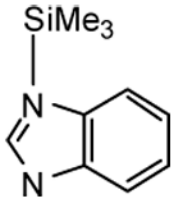
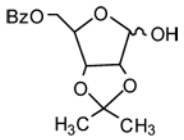
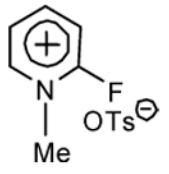
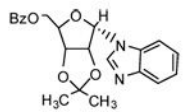
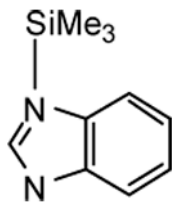
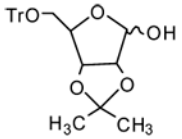
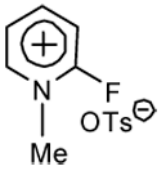
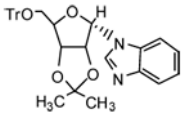
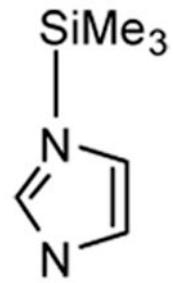
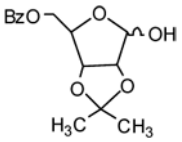
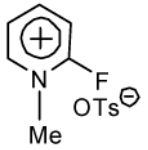
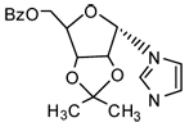
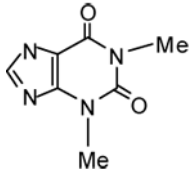
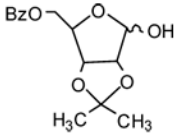
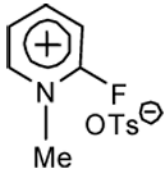
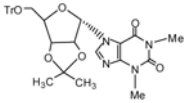
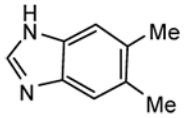
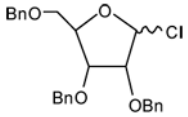
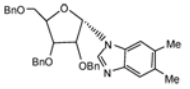
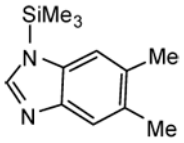
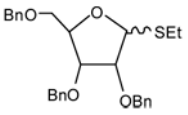
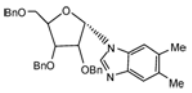
Base added to the medium	Analogues	Ref. no.
 Indoline	Did not incorporate	25
	Did not incorporate	25
	Did not incorporate	25
	Did not incorporate	25

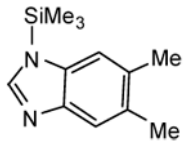
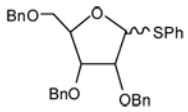
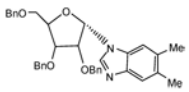
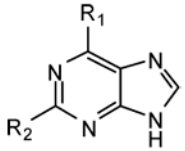
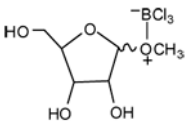
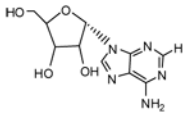
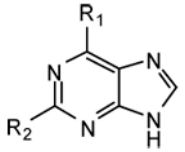
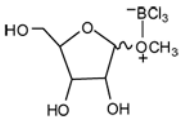
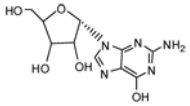
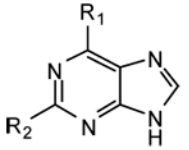
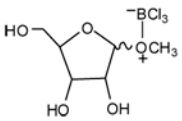
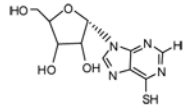
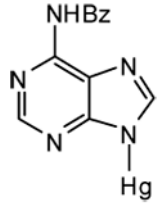
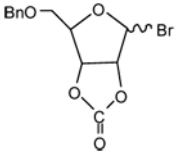
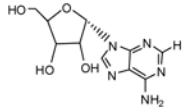
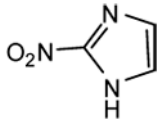
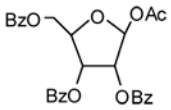
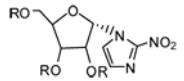
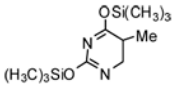
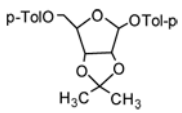
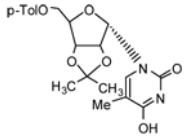
Table 2

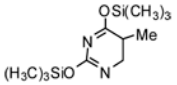
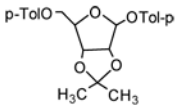
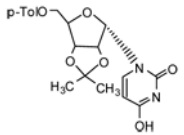
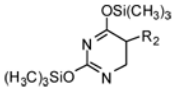
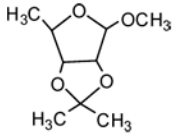
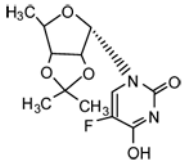
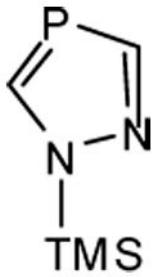
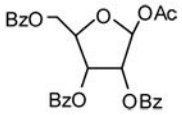
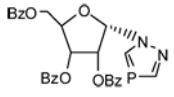
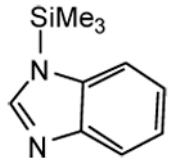
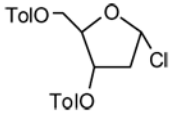
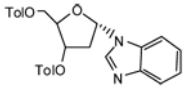
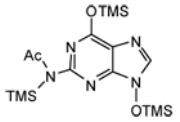
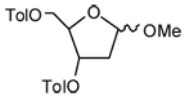
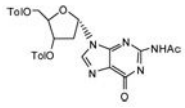
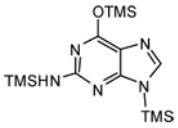
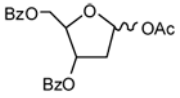
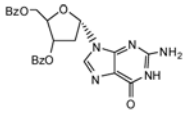
Summary of the glycosylation reactions, which produces maximum α-ribonucleosides

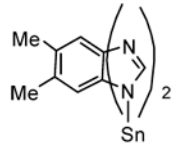
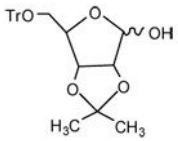
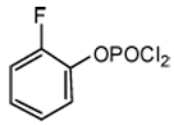
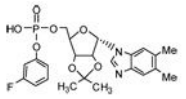
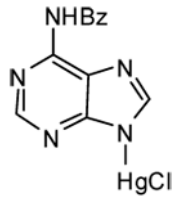
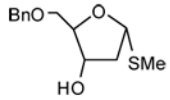
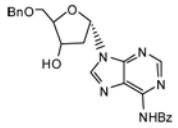
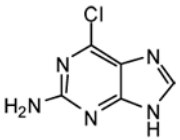
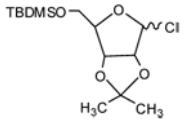
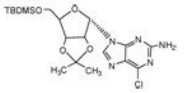
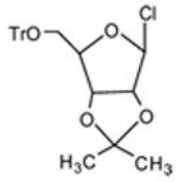
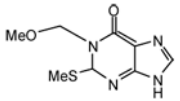
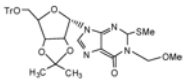
Base	Sugar	Catalyst	Nucleoside	(α) Yield s (%)	Reference
				75[a]	9
				65[a]	9
				65[a]	9
				60[a]	9

Base	Sugar	Catalyst	Nucleoside	(α) Yield s (%)	Reference
				96	12
				96	12
				96	12
				80[a]	12
				89	15

Base	Sugar	Catalyst	Nucleoside	(α) Yield s (%)	Reference
				70[a]	12
				86	15
				53	15
		none		66	35
		Mol. Sieve 4A, NBS			47

Base	Sugar	Catalyst	Nucleoside	(α) Yield s (%)	Reference
		Mol. Sieve 4A, NBS			47
		pyridine		55	48
		pyridine		55	48
		pyridine		55	48
		-		24	49
		$SnCl_4$, $Hg(CN)_2$		61	50
		TMSOTf		65	51

Base	Sugar	Catalyst	Nucleoside	(α) Yield s (%)	Reference
		TMSOTf		65	51
		TMSOTf		96	52
		TMSOTf			53
		$SnCl_4$			54
		$SnCl_4$			55
		KI, dibenzo-18-crown-6			56

Base	Sugar	Catalyst	Nucleoside	(α) Yield s (%)	Reference
					57
		$Cl(CH_2)_2Cl$			58
		K_2CO_3, NMP			59
		NaH, DMF			60

[a] isolated yields

Tetrahedron. Author manuscript; available in PMC 2009 January 1.

Table 3

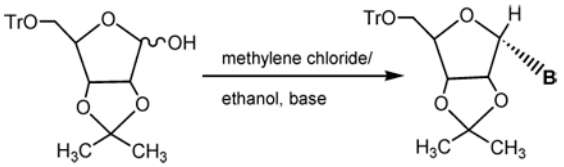
Isolated Yields and Crude Ratio's of α/*β*-5-Haloimidazole Ribonucleosides

***α/β*-Ribonucleoside**	**Crude ratio *α/β* (%)** [a]	**Isolated yields (%)**		**Total yields (%)**
		α	***β***	**α + *β***
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.

Table 4

Reaction conditions and yields of glycosylation of different bases with 2,3-O-(1-methylethylidene)-5-O-(triphenylmethyl)-D-ribofuranose

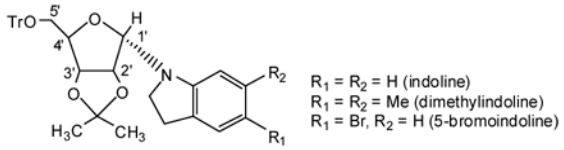


S. no.	Base (B)	solvent	rexn temp (°C)	rexn time (h)	yield[a]
1	indoline	ethanol	60–70	5–6	60
2	dimethylindoline	ethanol	60–70	4–5	65
3	5-bromoindoline	ethanol	60–70	6–7	55
4	indoline	methylene chloride	40	4	65
5	dimethylindoline	methylene chloride	40	4	70
6	5-bromoindoline	methylene chloride	40	5	60

[a] Isolated yields

Table 5

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



R_1 = R_2 = H (indoline)
R_1 = R_2 = Me (dimethylindoline)
R_1 = Br, R_2 = H (5-bromoindoline)

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 ^{1}H NMR values in (δ ppm)

[b] Prepared by direct glycosylation

Table 6

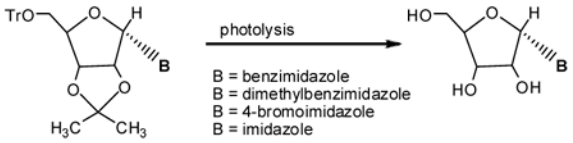
Comparison of dehydrogenation of protected nucleoside by using benzene/methylene chloride in presence of MnO_2 and molecular sieves

S.No.	Reactant	Solvent	Rexn. Temp. °C	Time (hr)	Yield(%)*
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

*Isolated yields

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 hr	95
2	α-benzimidazole[19]	0.5	20 min	75–80
3	α-5,6-DMBz[c]	0.05	1.0 hr	98
4	α-5,6-DMBz[c]	0.5	10–20 min	75
5	α-imidazole	0.05	50 min	86
6	α-4-bromoimidazole	0.05	1.0 hr	90
7	*β*-benzimidazole	0.05	1.0 hr	85
8	*β*-5,6-DMBz[c]	0.05	1.0 hr	85

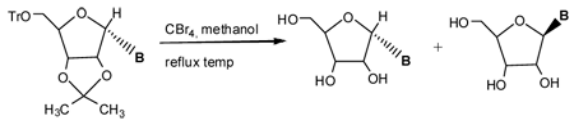
[a] Equiv. of CBr_4

[b] Isolated yields

[c] 5,6-DMBz = 5,6-dimethylbenzimidazole

Table 8

Reaction conditions and yields under reflux conditions in methanol and carbon tetrabromide:



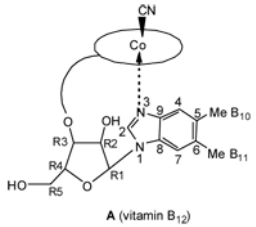
S. No.	Reactant (B)	$^{a}CBr_4$	Reaction time (hr)	Yield α-anomer (%)^{b}	Yield β-anomer (%)^{b}
1	α-benzimidazole	1–2	5–6	95	0
2	α-benzimidazole	0.5	10–12	85	5
3	α-5,6-DMBz[c]	2	5–6	75	0
4	α-5,6-DMBz[c]	0.5	10–12	70–80	5
5	α-imidazole	2	4–5	60–70	30
6	α-5-bromoimidazole	3	4–5	80	20

[a] Equiv. of CBr4

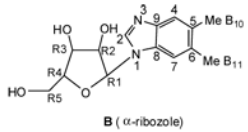
[b] NMR yields

Table 9

^{1}H and ^{13}C chemical shifts for included ribozole and free ribozole



A (vitamin B_{12})

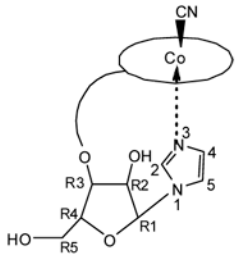


B (α-ribozole)

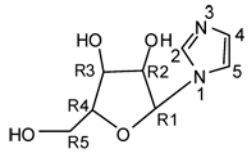
Atom	A (Vitamin B_{12}) $\delta^{1}H$	δ ^{13}C	$\delta^{1}H$	B (α-Ribozole) Δ Hz	Δ ^{13}C
B2	7.10				
B4	6.51	145.67	7.50		121.29
B7	7.28	119.3	7.37		135.23
B10	2.27	114.3	2.35		22.20
B11	2.26	22.20	2.34		21.91
R1	6.36	21.91	6.33	4.00	88.89
R2	4.28	89.8	4.55	4.40	74.17
R3	4.73	71.7	4.43	7.20	73.07
R4	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			

Table 10

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



A



B

Atom	A $\delta^{1}H$	A Δ Hz	A Δ ^{13}C	B $\delta^{1}H$	B Δ Hz	B 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

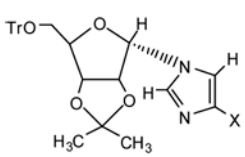
Table 11

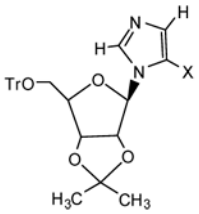
$^{1}H/^{13}C$ NMR chemical shifts of ribose sugar for α/*β* 5-haloimidazole ribonucleosides

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

Table 12

Methyl proton separation in (Δ ppm) for α/*β* imidazole/benzimidazole ribonucleosides





S. no.	compound	Me_1 (δppm)	Me_2 (δppm)	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_3OD
5	α-5-bromoimidazole	1.390	1.658	0.026	CD_3OD
6	α-5-iodoimidazole	1.280	1.356	0.076	CD_3OD
7	α-5- fluoroimidazole	1.277	1.310	0.033	DMSO-d_6
8	α-5-bromoimidazole	1.246	1.340	0.094	DMSO-d6
9	α-5-iodoimidazole	1.297	1.250	0.047	DMSO-d6
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_3OD
13	*β*-5-bromoimidazole	1.339	1.556	0.21	CD_3OD
14	α-benzimidazole	1.313	1.447	0.134	$CDCl_3$
15	α-benzimidazole	1.299	1.437	0.138	CD_2Cl_2
16	α-benzimidazole	1.279	1.412	0.133	Acetone-d6
17	α-benzimidazole	1.282	1.358	0.076	CD_3OD
18	α-benzimidazole	1.246	1.340	0.094	DMSO-d6
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_3OD
22	α- imidazole	1.225	1.377	0.15	DMSO-d6
23	*β*- imidazole	1.367	1.601	0.234	$CDCl_3$
24	*β*- imidazole	1.339	1.548	0.209	CD_3OD
25	*β*- imidazole	1.301	1.509	0.208	DMSO-d6
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_3OD
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_3OD
32	*β*- benzimidazole-5-OH	1.343	1.582	0.25	DMSO-d6

Table 13

Normalized proton-proton two spin-order values for the *α*- and *β*-ribonucleosides

Ribonucleosides	**Anomeric configuration**	$(H_z^{1'} H_z^{2'})/(H_{z0}^{1'}) * 100$
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