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# CHEMOSELECTIVE DEPROTECTION OF $\alpha$-INDOLE AND IMIDAZOLE RIBONUCLEOSIDES

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□ *A series of 2′,3′-isopropylidene and 5′-trityl-protected α-indole and α/β-benzimidazole and imidazole ribonucleosides were deprotected with different acids. Selectivity was achieved for 5′- versus 2′,3′- deprotection by using formic acid in the α-indole ribonucleoside series. Treatment of α-indole ribonucleosides with a mixture of formic acid and ether at room temperature afforded 2′,3′-deprotected α-ribonucleosides, whereas treatment of the α-benzimidazole ribonucleosides with the same acid afforded the 5′-deprotected ribonucleoside without any 2′, 3′-deprotected products. The structures of these ribonucleosides were elucidated with 2D (NOESY, COSY, and HMQC) NMR spectroscopy.*

**Keywords** Chemoselective; coenzyme $B_{12}$; biosynthesis; glycosylation

## INTRODUCTION

Semisynthesis of cobalamins[1–6] with altered axial nucleotide ligands requires cobyric acid[7] and an α-ribonucleotide[8] as precursors. Analogs of coenzyme $B_{12}$ (5′-deoxyadenosylcobalamin, AdoCbl) with altered axial nucleotide ligands, such as B3-deazaAdoCbl (Figure 1) are of interest as probes of the function, if any, of the axial nucleotide in the enzymatic activation of AdoCbl for carbon-cobalt bond homolysis[9,10]. Literature reports on the synthesis and deprotection of indole α-ribonucleosides are limited. Deprotection is a crucial step for these unstable α-ribonucleosides,[11–18] in order to retain the α-configuration and to achieve the synthesis of AdoCbl. Trityl (Tr), dimethoxytrityl (DMTr), and isopropylidene groups are commonly used for the protection of 5-hydroxyl and 2, 3-hydroxyl groups in both carbohydrate and nucleoside chemistry.[19] Strong protic

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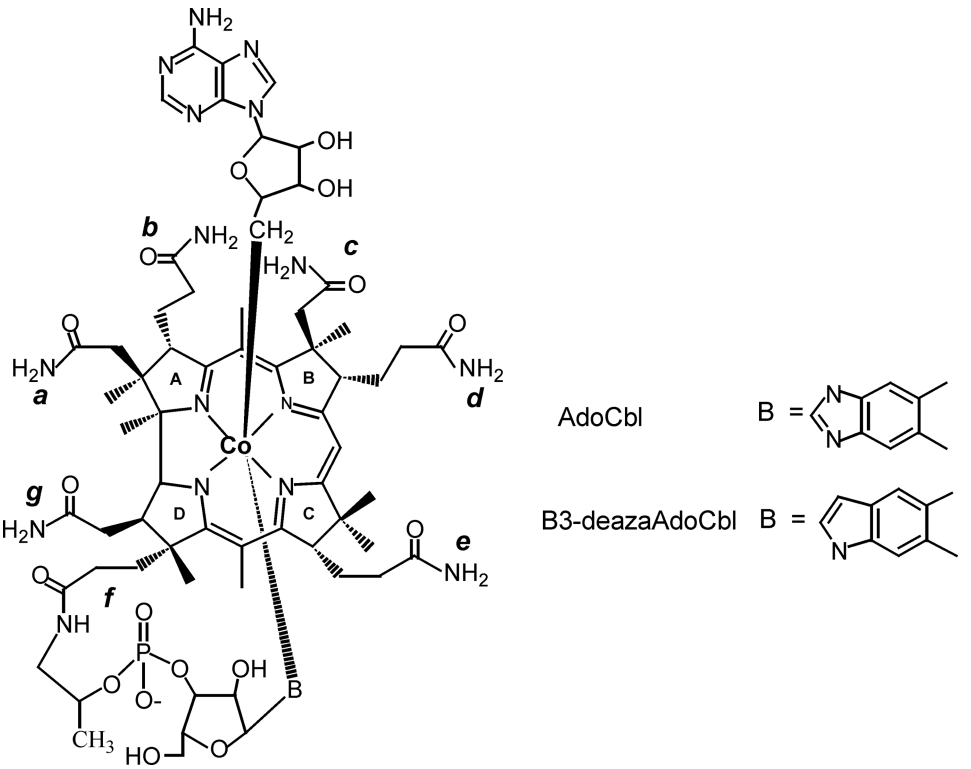
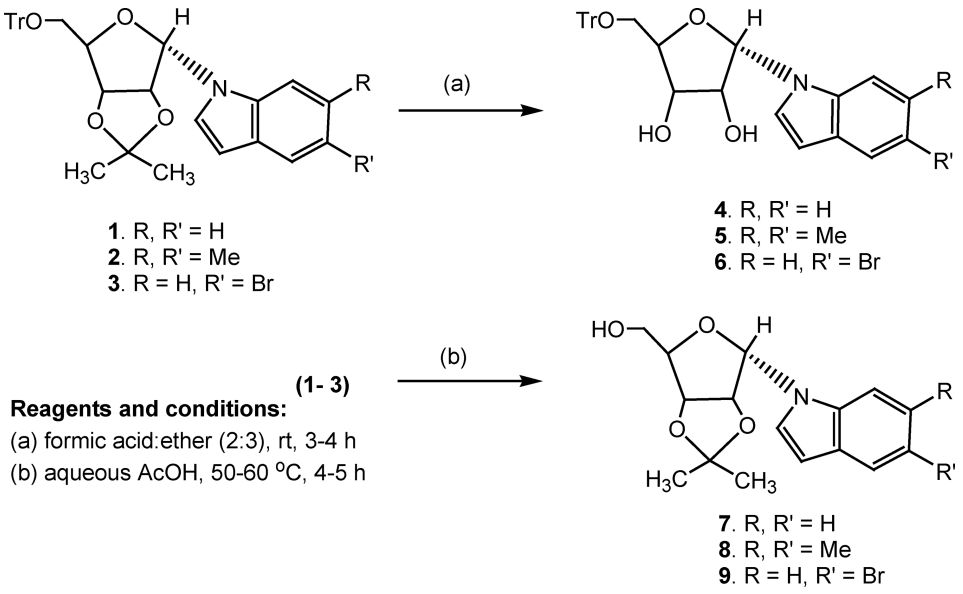



**FIGURE 1** Structure of AdoCbl (coenzyme $B_{12}$) and the analog B3-deaza AdoCbl.

and/or Lewis acids are generally used to hydrolyze these protecting groups with hydrochloric acid, formic acid[20] and trifluoroacetic acid (TFA)[21] typically being used for the deprotection of trityl groups. However under these conditions, ribonucleosides with the $\alpha$-anomeric configuration may undergo deglycosylation or decomposition. Dimethylindole, indole and other $\alpha$-ribonucleosides[13–15] when treated with neat TFA or heated for prolonged periods in acetic acid, give a charred product without any of the desired deprotected 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. In this report, we describe the selective deprotection of trityl and isopropylidene groups using different acids in organic solvents.

## RESULTS AND DISCUSSION

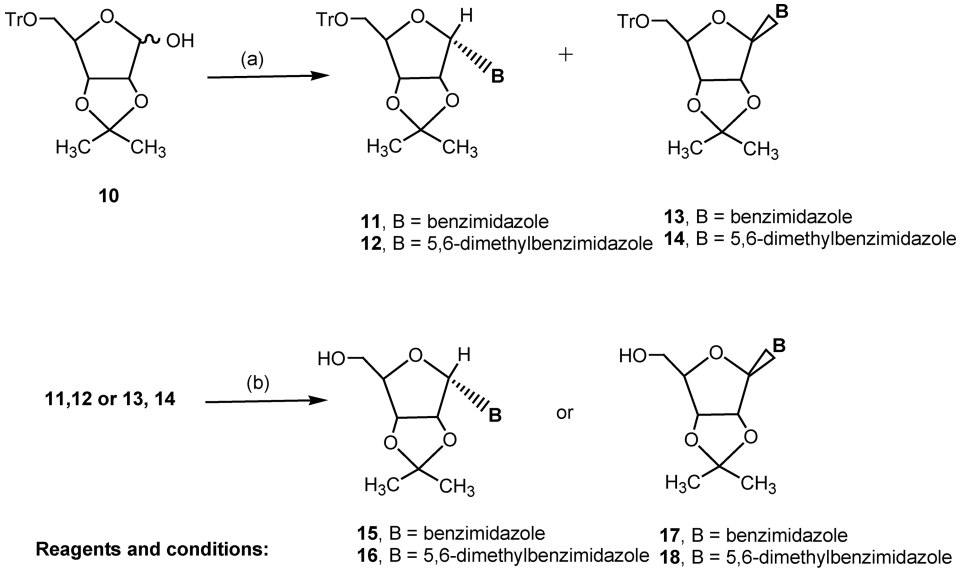
The protected $\alpha$-ribonuclesides were prepared by published methods, coupling trimethylsilyl protected indolines and protected D-ribose




**SCHEME 1** Deprotection of α-indole ribonucleosides.

using 2-fluoromethylpyridinium tosylate as a condensing reagent[22] or by direct glycosylation.[14] 2D NMR and X-ray crystallography confirmed the anomeric configuration of these indoline ribonucleosides.[15] Protected indole ribonucleosides[13] (**1–3**, Scheme 1) were prepared from the corresponding indoline α-ribonucleosides[14,15] by manganese dioxide oxidation[13] at elevated temperature. Benzimidazole, 5,6-dimethylbenzimidazole and imidazole α-ribonucleosides[15] (**11** & **12**) and (**13** & **14**) were similarly prepared using 2-fluoromethyl pyridinium tosylate as a condensing reagent[22] (Scheme 2). These glycosylation reactions produce mixtures of α- (70–85%) and β-ribonucleosides (15–30%).

Upon treatment with formic acid/ether (2:3) at room temperature (Scheme 1) the indole ribonucleosides afforded the 2′,3′-deprotected indole ribonucleoside 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 without affecting the isopropylidene group or causing deglycosylation. At higher temperature and longer time we observed deglycosylation and charring without any desired product. While it is known that 2′,3′-O-isopropylidene-β-D-ribofuranosyl-2,5,6-trichloroindole[23] and other halogenated indole ribonucleosides produce 2′,3′-deprotected indole ribonucleosides upon prolonged heating with acetic acid and water (4:1), for the current α-indole ribonucleosides we do not observe any 2′,3′-deprotected products. Instead,




**SCHEME 2** Preparation and deprotection of α/ß-benzimidazole/imidazoleribunucleosides.

prolonged heating of these α-indole ribonucleosides at higher temperature in acetic acid produced only charring.

The 1-(2,3-O-isopropylidene-5′-O-triphenylmethyl-α-D-ribofuranosyl)-benzimidazole, and 5,6-dimethylbenzimidazole ribonucleosides (**11** & **12**), however, react differently (Scheme 2). The trityl group can be easily removed by treating the protected ribonucleoside with formic acid/ether, heating with aqueous acetic acid, or using 1% TFA in methylene chloride, without affecting the isopropylidene.

A series of protected α and β ribosides were prepared by the Mukaiyama[22] method and isolated in pure form and thoroughly characterized by two-dimentional NMR techniques (COSY, HMQC, and NOESY). All protected α-ribonucleosides have a NOE between the isopropylidene methyl protons and imidazole proton, whereas the β-anomer does not have a NOE between these protons. The methyl protons of the isopropylidene had a NOE with the imidazole proton in 5′-deprotected α-benzimidazole ribonucleoside **15,** but the β-benzimidazole 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, **15,** caused a 10% positive NOE enhancement of the imidazole proton, and the precursor

**11** (protected ribonucleoside) shows a 12% NOE enhancement upon irradiation of the methyl protons. The signals for the methyl protons of the isopropylidene in the benzimidazole ribonucleoside (2′,3′-O-isopropylidene-α-D-ribofuranosyl) benzimidazole, **15,** were visible at δ 1.279 and 1.382 ppm (d = 0.103). The anomeric proton signal appeared at δ 6.33 ppm with a J value of 4.5 Hz, which further confirms the α-configuration of the ribonucleoside, whereas the anomeric proton signal for compound **17** was at δ 6.07 ppm with J value of 3.2 Hz and a methyl proton separation of 0.269 ppm. Similarly, the deprotected indole ribonucleosides also showed the same NMR pattern as the protected α-ribonucleosides.

## EXPERIMENTAL SECTION

All reactions were carried out in oven or flame-dried glassware under a nitrogen atmosphere. Solvents were distilled prior to use. Dichloromethane, benzene and ether were distilled from calcium hydride. Purification of reaction products was carried out by flash chromatography using silica gel (230–400 mesh). All reagents were commercially available and used without further purification. All reactions were monitored by thin-layer chromatography (TLC) using Merck silica gel 60, F-254. Column chromatography was conducted using flash silica gel.

### Physical Measurements

$^{1}$H NMR and $^{13}$C NMR spectra were recorded on a Varian INOVA-500 or VXR-400 NMR spectrometers using the residual proton resonance of the solvent as an internal reference at 25°C. Two-dimensional NMR (COSY, NOESY) spectra were obtained at 25°C on a Varian INOVA-500 NMR spectrometer using TMS as an internal reference. The multiplicities of the $^{13}$C NMR signals were determined by the HMQC and DEPT technique. Mass data were obtained at University of Illinois using a micromass Quattro-I mass spectrometer.

**1-(5-O-Triphenylmethyl-α-D-ribofuranosyl)indole (4):** A mixture of the protected ribonucleoside **1**[13] (0.700 g, 1.3 mmol) in ether:formic acid (3:2) (10 mL) was stirred for 3–4 hours at room temperature while TLC (ethyl acetate:hexane 1:1) was used to monitor the progress of the reaction. Upon completion, the solvent was removed on a rotary evaporator, the residue was dissolved in ethyl acetate, and washed with saturated sodium bicarbonate solution. The ethyl acetate layer was concentrated and the residue was purified by flash chromatography. Yield: 60%; $R_f$. 0.2; white solid; $^{1}$H NMR (DMSO-$d_6$): δ 3.57 (dd, J = 2.0, 5.5 Hz, 1H, 5′), 3.70 (d, J = 7.7 Hz, 1H, 5″), 3.84 (bt, 1H, 2′), 4.0 (bs, 1H), 5.0 (d, J = 3.4 Hz, 3′), 5.49 (d, J = 9.5 Hz, 1H, 1′), 6.47 (d, J = 8.0 Hz, 1H, Ar), 6.70 (t, J = 7.5 Hz, 1 H, Ar), 6.93 (s, 1H, Ar), 7.1 (d, J = 7.5 Hz, 6H, Ar), 7.2 (t, 3H,

trityl), 7.27 (t, J = 7.7 Hz, 6H, trityl), 7.44 (d, 1H, J = 8.0 Hz, Ar); $^{13}$C NMR (DMSO-$d_6$): 65.0, 66.64, 68.45, 71.43, 81.98 (C1′), 110.8 (CH), 119.0 (CH), 121.1 (CH), 121.7 (CH), 121.8 (Cquat), 126.0 (CH), 126.48, 127.48 (CH), 128.0 (Cquat), 130.1 (CH), 137.25, 145.9 (Cquat); HRMS: *m/z*: 492.2166 (calcd for $C_{32}H_{30}NO_4$; 492.2174) (M + H); MS: (FAB) *m/z* 492, 394, 324, 243.

**1-(2, 3-isopropylidene-α-D-ribofuranosyl)5,6-dimethylindole (8):** Compound **2**[13] (150 mg, 0.26 mmol) was dissolved in aqueous acetic acid (5 mL) and heated for 5 hours at 50–60°C in a oil bath. After complete conversion of the starting material, the reaction mixture was cooled and then concentrated to dryness to give a residue. The residue was purified by flash column chromatography (benzene:ether; 50:50) to give 70 mg of compound **8** as a viscous oil. Yield: 80%; $R_f$. 0.7; $^1$H NMR ($CDCl_3$): δ 1.38 (s, 3H, $CH_3$, isopropylidene), 1.64 (s, 3H, $CH_3$, isopropylidene), 2.35 (s, 3H, $CH_3$), 2.40 (s, 3H, $CH_3$), 3.73 (dd, J = 4.0, 8.0Hz, 1H, 5′), 3.82 (dd, J = 3.24, 8.9Hz, 1H, 5′), 4.22 (q, 1H, CH, 4′), 4.93–4.95 (m, 1H, CH, 3′), 5.03–5.05 (m, 1H, CH, 2′), 6.06 (d, J = 3.5 Hz, 1H, CH, 1′), 6.46 (d, J = 3.0 Hz, 1H, indole), 7.10 (d, J = 3.0 Hz, 1H, indole), 7.27 (s, 1H, Ar), 7.39 (s, 1H, Ar); $^{13}$C NMR ($CDCl_3$): δ 19.97 ($CH_3$), 20.70 ($CH_3$), 25.36 ($CH_3$), 27.26 ($CH_3$), 62.52 ($CH_2$, 5′), 80.18 (C 3′), 84.03 (C 2′), 84.83 (C 4′), 91.15 (C 1′), 103.13 (CH, Ar), 110.52 (CH), 114.70 (Cquat, isoprop), 121.25 (CH), 123.72 (CH), 126.01 (Cquat), 127.35 (Cquat), 130.75 (Cquat), 146.22 (Cquat); HRMS: *m/z*: 318.1704 (calcd for $C_{18}H_{24}NO_4$; 318.1704) (M + H).

**1-(2,3-O-isopropylidene-α-D-ribofuranosyl)benzimidazole (15):** The benzimidazole ribonucleoside[15,17] **11**, was similarly deprotected as described for the indole nucleoside **4** using either the mixture of (formic acid:ether) or aqueous acetic acid (method described for the compound **8**) Yield: 80–90%; $R_f$. 0.3; White foam; $^1$H NMR ($CDCl_3$): δ 1.279 (s, 3H, $CH_3$, isopropylidene), 1.382 (s, 3H, $CH_3$, isopropylidene), 3.85 (dd, J = 3.5, 8.5 Hz, 1H, 5′), 3.96 (dd, J = 2.0, 9.5 Hz, 1H, 5″), 4.42 (bt, 1H, CH, 4′), 4.92 (t, J = 6.5 Hz, 1H, CH, 2′), 5.01 (d, J = 6 Hz, 1H, CH, 3′), 6.33 (d, J = 4.5 Hz, 1H, CH, 1′), 7.21–7.28 (m, 3H, Ar), 7.76 (d, J = 7.5 Hz, 1H, Ar), 8.24 (s, 1H, imidazole); $^{13}$C NMR ($CDCl_3$): δ 24.18 ($CH_3$), 25.65 ($CH_3$), 63.87 ($CH_2$, 5′), 80.06 (C 2′), 82.35 (C 3′), 83.45 (C 4′), 87.21 (C 1′), 109.90 (CH, Ar), 113.34 (Cquat, isoprop), 119.61 (CH), 122.47 (CH), 123.06 (CH), 132.69 (Cquat), 142.30 (CH), 142.58 (Cquat); HRMS: *m/z*: 291.1344 (calcd for $C_{15}H_{19}N_2O_4$; 291.1343) (M + H); MS: (FAB) *m/z* 291, 195, 152, 119.

**1-(2, 3-O-isopropylidene-β-D-ribofuranosyl)benzimidazole (17):** Yield: 80–90%; $R_f$. 0.3; White crystalline solid; $^1$H NMR ($CDCl_3$): δ 1.40 (s, 3H, $CH_3$, isopropylidene), 1.67 (s, 3H, $CH_3$, isopropylidene), 3.85 (dd, J = 2.83, 8.91Hz, 1H, 5′), 4.01 (dd, J = 2.43, 9.72Hz, 1H, 5″), 4.51 (d, 1H, CH, 4′), 4.65 (bs, 1H, OH), 4.98–4.99 (m, 1H, CH, 2′), 5.05 (dd, J = 2.43, 3.64 Hz, 1H, CH, 3′), 6.07 (d, J = 3.24 Hz, 1 H, CH, 1′), 7.29–7.32 (m, 2H, Ar), 7.52 (d, J = 7.29 Hz, 1H, Ar), 7.75 (d, 1H, J = 6.89Hz, 1H, Ar), 8.39 (s, 1H,

imidazole); $^{13}$C NMR ($CDCl_3$): δ 25.25 ($CH_3$), 27.28 ($CH_3$), 62.05 ($CH_2$, 5′), 81.50 (C 3′), 85.36 (C 2′), 86.52 (C 4′), 92.81 (C 1′), 110.46 (CH, Ar), 114.18 (Cquat, isoprop), 119.87 (CH), 122.82 (CH), 123.44 (CH), 132.41 (Cquat), 142.83 (CH), 143.33 (Cquat); HRMS: *m/z*: 291.1346 (calcd for $C_{15}H_{19}N_2O_4$; 291.1343) (M + H); MS: (FAB) *m/z* 291, 195, 152, 119.

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