# Chemical Synthesis and Properties of Analogs of Adenosylcobalamin†

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ABSTRACT: A series of analogs of adenosylcobalamin differing in the nucleoside moiety has been prepared by reacting cob(I)alamin with 5′-deoxy-5′-chloronucleosides. The chloronucleosides were prepared directly from the parent nucleosides by the procedure of Kikugawa and Ichino (*Tetrahedron Lett.*, 87 (1971)). The physical and chemical properties of these analogs have been determined.

Almost immediately after the structure of adenosylcobalamin had been elucidated by X-ray crystallography (Lenhert and Hodgkin, 1961), methods were developed for the chemical synthesis of the unique carbon–cobalt bond (Müller and Müller, 1962; Johnson *et al.*, 1963). These syntheses involved the displacement of the tosylate ion from a 2′,3′-*O*-isopropylidene-5′-*p*-toluenesulfonyl ribonucleoside by the powerful nucleophile cob(I)alamin, followed by treatment of the resulting cobalamin with acid to remove the isopropylidene protecting group. Unfortunately the rather vigorous conditions necessary for the removal of the isopropylidene group gave rise to undesirable side products (Morley *et al.*, 1968). To avoid the formation of these by-products other reagents for the protection of the 2′,3′-*cis*-glycol groups were introduced (Schmidt and Huennekens, 1967; Morley and Hogenkamp, 1968; Yurkevich *et al.*, 1969; Law *et al.*, 1971). Using these procedures numerous analogs of adenosylcobalamin differing in the base and/or sugar moiety have been prepared (Hogenkamp *et al.*, 1971; Borodulina-Shvets *et al.*, 1973; Rudakova *et al.*, 1973).

Various analogs have also been prepared without the aid of a protecting group, but generally in much lower yield due to the tendency of the unprotected 5′-*p*-toluenesulfonyl ribonucleoside to cyclize (Zagalak and Pawelkiewicz, 1965a,b).

Adenosylcobalamin and some of its analogs have been synthesized by reacting cob(I)alamin with suitably protected 5′-iodonucleosides (Murakami *et al.*, 1966, 1967) or 5′-methanesulfonyl nucleosides (Borodulina-Shvets *et al.*, 1973).

A very convenient method for the preparation of 5′-halonucleosides has been described by Kikugawa and Ichino (1971). Thionyl chloride or thionyl bromide mixed with hexamethylphosphoramide was found to specifically halogenate the 5′ position of ribonucleosides and thus no prior blocking of the *cis*-hydroxyl functions was required. This method is ideally suited for the synthesis of analogs of adenosylcobalamin containing precious nucleosides such as aristeromycin or for the synthesis of adenosylcobalamin labeled with isotopes of hydrogen or carbon in the adenosyl moiety. With this procedure a series of 5′-deoxy-5′-chlororibonucleosides has been prepared and these nucleosides have been used for the partial synthesis of analogs of adenosylcobalamin. Some physical and chemical properties of these analogs are also reported.

## Materials and Methods

*Materials.* Nucleosides were purchased from the commercial suppliers indicated: adenosine, 2′-deoxyadenosine, purine ribonucleoside (nebularine), inosine and cytidine, Sigma Chemical Co.; tubercidin, CalBiochem; guanosine and 9-β-D-arabinofuranosyladenine,[1] P-L Biochemicals. Aristeromycin (the carbocyclic analog of adenosine) was a generous gift from Dr. T. Kusaka, Takeda Chemical Industries, Ltd.; 4′-thioadenosine was a gift from Dr. L. Goodman, Stanford Research Institute; 3′-amino-3′-deoxyadenosine was obtained from Dr. N. N. Gerber, Rutgers University and formycin from Dr. R. L. Blakley, University of Iowa. Cyanocobalamin was purchased from Sigma Chemical Co. The following compounds were synthesized by published procedures: aquocobalamin (Hogenkamp and Rush, 1968); adenosylcobalamin and isopropylideneadenosylcobalamin (Hogenkamp and Pailes, 1968); 9-(3′-hydroxypropyl)adenine and 9-(4′-hydroxybutyl)adenine (Ikehara *et al.*, 1961); 3-β-D-ribofuranosyladenine (Leonard and Laursen, 1965); 9-β-D-arabinofuranosyladenine (Glaudemans and Fletcher, 1963); 9-β-D-xylofuranosyladenine (Baker and Hewson, 1957); L-ribose (Walker and Hogenkamp, 1974); 9-β-L-ribofuranosyladenine (Ryan and Acton, 1968); 9-(5′,6′-dideoxy-β-D-*ribo*-heptofuranosyl)-adenine (Walker *et al.*, 1973) and 1-β-D-ribofuranosylbenzimidazole (Davoll and Brown, 1951).

*Methods.* Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Melting points were measured on a hot stage equipped with a microscope, and are not corrected. Nuclear magnetic resonance (nmr) spectra were obtained using Varian HA-100 and HR-220 spectrometers, chemical shifts are recorded in parts per million down field from an internal standard of tetramethylsilane. Ultraviolet and visible spectra were recorded on a Cary Model 15 spectrophotometer. Other absorbance measurements were made with a Zeiss PMQ II spectrophotometer. Molar extinction coefficients of the cobalamins are based on $\epsilon_{365} = 30.8 \times 10^3$ $M^{-1}$ $cm^{-1}$ for dicyanocobalamin (Friedrich, 1964). The cobalamins were converted to dicyanocobalamin by photolysis in the presence of 0.1 M KCN. Descending chromatography on Whatman No. 1 paper was conducted with the following solvent systems: solvent I, 84:16 1-butanol–water; II, 50:36:14 *sec*-butyl alcohol–water–ammonium hydroxide; III, 50:15:35 1-butanol–ethanol–water; IV, 37:26:37 1-butanol–

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[1] Abbreviations used are: 3-isoadenosine, 3-β-D-ribofuranosyladenine; *ara*-adenosine, 9-β-D-arabinofuranosyladenine; *xylo*-adenosine, 9-β-D-xylofuranosyladenine.

isopropyl alcohol–water. Nucleosides on paper chromatograms were detected by their absorption of ultraviolet light.

*Partial Synthesis of Adenosylcobalamin.* To a solution of 0.15 ml of thionyl chloride in 1 ml of hexamethylphosphoramide was added 95 mg (0.35 mmol) of adenosine and the reaction mixture was stirred with exclusion of moisture at room temperature for 12 hr. The reaction mixture was then added to 5 ml of ice–water and the aqueous solution was applied to a column (2 × 2 cm) of Dowex 50-X2 ($H^+$) (200–400 mesh). The column was washed with water and then treated with 1 M ammonium hydroxide. The fractions containing 5′-deoxy-5′-chloroadenosine were combined and evaporated to dryness. Paper chromatography in solvent I showed that the desired nucleoside was homogeneous ($R_{adenosine}$ = 1.85). The 5′-deoxy-5′-chloronucleoside was then reacted with cob(I)-alamin, from 700 mg (0.46 mmol) of aquocobalamin reduced with 100 mg of sodium borohydride in the presence of 7 mg of cobaltous chloride by procedures previously described (Hogenkamp *et al.*, 1971; Dolphin, 1971). The reaction mixture was acidified to pH 3.0 with 1 N hydrochloric acid and applied to a column (2.5 × 35 cm) of Dowex 50-X2 (200–400 mesh, pH 3.0). The column was washed with water and adenosylcobalamin was eluted with 0.1 N sodium acetate (pH 6.4). Adenosylcobalamin was isolated as described before (Hogenkamp and Pailes, 1968) and crystallized from 90% acetone with a yield of 434 mg (77% from adenosine). It was chromatographically homogeneous in solvents II, III, and IV (Table I).

*Partial Synthesis of Analogs of Adenosylcobalamin.* Using an identical procedure analogs were prepared containing the following nucleosides: 9-(3′-hydroxypropyl)adenine, 9-(4′-hydroxybutyl)adenine, 9-(5′,6′-dideoxy-β-D-*ribo*-heptofuranosyl)adenine, 9-β-L-ribofuranosyladenine, 3-isoadenosine, aristeromycin, tubercidin, *ara*-adenosine, nebularine, inosine, cytidine, and 1-β-D-ribofuranosylbenzimidazole. While most of the analogs could be eluted from the Dowex 50-X2 (pH 3) column with 0.1 M sodium acetate (pH 6.4), the elution of the cobalamins containing tubercidin (p$K$ = 5.3) and 3-isoadenosine (p$K$ = 5.5) required 0.1 M Tris-HCl (pH 7.6). The cobalamin analogs derived from the antibiotics nebularine, tubercidin, and aristeromycin have so far resisted crystallization from aqueous acetone. These preparations were evaporated to dryness and isolated as a glass. All cobalamins were homogeneous on paper chromatography in solvents II, III, and IV (Table I). While most analogs were obtained in excellent yields (70–85% based on the nucleoside), the yield of 3-isoadenosylcobalamin was only 30%. The direct halogenation procedure was not suitable for the preparation of cobalamin analogs of the following nucleosides: guanosine, 2′-deoxyadenosine, 2′-deoxyuridine, thymidine, 4′-thioadenosine, 3′-amino-3′-deoxyadenosine, formycin, and *xylo*-adenosine.

TABLE I: Paper Chromatographic Properties of Cobalamin Analogs.

| Nature of the Nucleoside | $R_{cyanocobalamin}$ II | III | IV |
|---|---|---|---|
| Adenosine | 0.78 | 0.84 | 0.76 |
| 2′,3′-*O*-Isopropylideneadenosine | 1.62 | 1.42 | 1.72 |
| 9-(3′-Hydroxypropyl)adenine | 0.85 | 1.03 | 1.02 |
| 9-(4′-Hydroxybutyl)adenine | 1.00 | 1.10 | 1.12 |
| 9-(5′,6′-Dideoxy-β-D-*ribo*-heptofuranosyl)adenine | 0.90 | 1.05 | 1.00 |
| Nebularine | 0.78 | 0.95 | 0.95 |
| Tubercidin | 0.83 | 0.69 | 0.74 |
| 3-β-D-Ribofuranosyladenine | 0.63 | 0.78 | 0.74 |
| 9-β-L-Ribofuranosyladenine | 0.57 | 0.70 | 0.72 |
| Aristeromycin | 0.68 | 0.75 | 0.74 |
| 9-β-D-Arabinofuranosyladenine | 0.60 | 0.61 | 0.64 |
| Inosine | 0.47 | 0.53 | 0.52 |
| Cytidine | 0.55 | 0.58 | 0.65 |
| 1-β-D-Ribofuranosylbenzimidazole | 1.44 | 1.48 | 1.43 |

*Halogenation of 2′-Deoxyribonucleosides and of ara-Adenosine.* 2′,3′,5′-TRIDEOXY-3′,5′-DICHLOROADENOSINE. To a solution of 1.5 ml of thionyl chloride in 10 ml of hexamethylphosphoramide was added 1 g (4 mmol) of deoxyadenosine and the reaction mixture was stirred with exclusion of moisture at room temperature for 12 hr. To the reaction mixture was then added 12 ml of 1 M $K_2HPO_4$ and applied to a column (2 × 4 cm) of Dowex 50-X2 ($H^+$) (200–400 mesh). The column was washed with water and treated with 1 M ammonium hydroxide. The product crystallized during concentration of the eluate *in vacuo* at 45° and was recrystallized from water–methanol; yield 610 mg (53%); mp 174–176° dec; $\lambda_{max}^{MeOH}$ 259 nm (ε 14,400); nmr ($d_5$-pyridine) 2.98 (oct, 1, $J_{2a',2b'}$ = 16 Hz, $J_{2a',3'}$ = 2 Hz, $C_{2a'}H$), 3.22 (oct, 1, $J_{2b',3'}$ = 6 Hz, $C_{2b'}H$), 3.97 (q, 1, $J_{5a',5b'}$ = 11 Hz, $J_{4',5a'}$ = 6.5 Hz, $J_{4',5b'}$ = 6.5 Hz, $C_{5a'}$ or $C_{5b'}$ H), 4.05 (q, 1, $C_{5a'}$ or $C_{5b'}H$), 4.52 (sx, 1, $J_{3',4'}$ = 3.5 Hz, $C_{4'}H$), 4.86 (oct, 1, $C_{3'}H$), 6.66 (q, 1, $J_{1'2a'}$ = 3.5 Hz, $J_{1',2b'}$ = 8 Hz, $C_{1'}H$), 8.18 (s, 2, $NH_2$), 8.55 (s, 1, $C_2H$ or $C_8H$), 8.67 ppm (s, 1, $C_2H$ or $C_8H$). *Anal.* Calcd for $C_{10}H_{11}Cl_2N_5O$: C, 41.66; H, 3.82; Cl, 24.65; N, 24.31. Found: C, 41.65; H, 3.60; Cl, 24.49; N, 24.09.

2′,3′,5′-TRIDEOXY-3′,5′-DICHLOROURIDINE. 2′-Deoxyuridine (850 mg, 3.7 mmol) was dissolved in a mixture of 10 ml of hexamethylphosphoramide and 1 ml of thionyl chloride. After 12 hr the reaction mixture was added to 50 ml of chloroform, extracted four times with water, dried over sodium sulfate, and evaporated *in vacuo*. Crystallization from methanol–water gave 474 mg (48%) of tan crystals, mp 217–220°; $\lambda_{max}^{MeOH}$ 261 nm (ε 10,000); nmr ($d_5$-pyridine) 2.53 (oct, 1, $J_{2a',2b'}$ = 16 Hz, $J_{2a',3'}$ = 1 Hz, $C_{2a'}H$), 3.12 (oct, 1, $J_{2b',3'}$ = 6.5 Hz, $C_{2b'}H$), 3.96 (q, 1, $J_{4',5a'}$ = 6 Hz, $C_{5a'}H$), 3.98 (q, 1, $J_{4',5b'}$ = 6 Hz, $C_{5b'}H$), 4.43 (sx, 1, $C_{4'}H$), 4.79 (oct, 1, $J_{3',4'}$ = 3.5 Hz, $C_{3'}H$), 5.87 (d, 1, $J_{5,6}$ = 8 Hz, $C_5H$), 6.43 (q, 1, $J_{1',2a'}$ = 3 Hz, $J_{1',2b'}$ = 8 Hz, $C_{1'}H$), 7.85 ppm (d, 1, $C_6H$). *Anal.* Calcd for $C_9H_{10}Cl_2N_2O_3$: C, 40.75; H, 3.77; Cl, 26.79; N, 10.57. Found: C, 40.99; H, 3.94; Cl, 26.49; N, 10.76.

3′,5′-TRIDEOXY-3′,5′-DICHLOROTHYMIDINE. Thymidine (1.0 g, 4.1 mmol) was dissolved in a mixture of 10 ml of hexamethylphosphoramide and 1 ml of thionyl chloride. After 12 hr the reaction mixture was worked up as described for the deoxyuridine reaction. The product was crystallized from methanol–water; yield 715 mg (62%); mp 145–147°; $\lambda_{max}^{MeOH}$ 266 nm (ε 9920); nmr ($d_5$-pyridine) 1.98 (d, 3, $C_5CH_3$), 2.53 (oct, 1, $J_{2a',2b'}$ = 16 Hz, $J_{2a',3'}$ = 1.3 Hz, $C_{2a'}H$), 3.10 (oct, 1, $J_{2b',3'}$ = 6.5 Hz, $C_{2b'}H$), 3.97 (q, 1, $J_{4',5'}$ = 6 Hz, $C_{5a'}H$), 3.98 (q, 1, $C_{5b'}H$), 4.39 (sx, 1, $J_{3',4'}$ = 3.5 Hz, $C_{4'}H$), 4.80 (oct, 1, $C_{3'}H$), 6.52 (q, 1, $J_{1',2a'}$ = 3.7 Hz, $J_{1',2b'}$ = 8 Hz, $C_{1'}H$), 7.67 ppm (d, 1, $C_6H$). *Anal.* Calcd for $C_{10}H_{12}Cl_2N_2O_3$: C, 43.01; H, 4.30; Cl, 25.45; N, 10.04. Found: C, 43.02; H, 4.26; Cl, 25.27; N, 9.91.

5′-DEOXY-5′-CHLORO-*ara*-ADENOSINE. To a solution of 0.5 ml of thionyl chloride in 5 ml of hexamethylphosphoramide was added 500 mg (1.8 mmol) of *ara*-adenosine and the reaction mixture was stirred with exclusion of moisture at room temperature for 12 hr. The reaction mixture was then added to 10 ml of ice–water and the aqueous solution was applied to a

TABLE II: Absorption Spectra of Adenosylcobalamin and Some of Its Analogs.

| Nature of the Nucleoside | 0.1 N HCl | 0.1 M Phosphate Buffer (pH 7.0) |
|---|---|---|
| Adenosine | 266 287 306 380 458<br>(41.1)(23.9)(22.2)(8.8)(9.4) | 262 290 318 341 376 522<br>(35.1)(18.2)(13.0)(12.8)(11.0)(8.0) |
| 9-(5′,6′-Dideoxy-β-D-*ribo*-hepto-furanosyl)adenine | 264 286 304 381 455<br>(39.2)(22.3)(26.3)(9.2)(8.8) | 264 290 317 344 375 510<br>(31.2)(17.4)(14.3)(12.4)(9.7)(9.4) |
| Nebularine | 264 285 304 378 458<br>(36.8)(23.7)(21.9)(7.6)(9.1) | 265 280 290 318 340 377 522<br>(30.8)(20.7)(18.6)(12.6)(12.4)(10.4)(8.2) |
| Tubercidin | 266 275 286 350 460<br>(44.8)(41.5)(37.3)(14.7)(10.4) | 266 278 348 480 522<br>(40.1)(36.8)(15.7)(7.8)(8.7) |
| 3-β-D-Ribofuranosyladenine | 267 277 304, 317, 330 349 462<br>(40.9)(39.3) S (15.1)(9.3) | 268 280 290 350 377 522<br>(32.0)(31.2) S (15.8) S (8.6) |
| 9-β-L-Ribofuranosyladenine | 264 287 306 381 455<br>(42.7)(24.3)(24.0)(9.5)(10.6) | 264 290 317 342 373 520<br>(37.5)(19.9)(14.2)(13.8)(12.1)(9.3) |
| Aristeromycin | 264 286 305 382 457<br>(45.4)(23.9)(24.6)(7.9)(8.6) | 263 290 318 338 378 523<br>(38.0)(18.4)(13.4)(12.6)(8.9)(8.1) |
| 1-β-D-Ribofuranosylbenzimidazole | 265 277 287 304 379 458<br>(34.8)(28.8)(23.6)(22.2)(8.2)(9.6) | 266 281 289 316 339 376 522<br>(26.0)(23.6)(19.1)(13.5)(13.5)(11.3)(8.7) |
| Cytidine | 264 276 285 305, 315 375 458<br>(36.5)(34.0)(32.9) S (8.8)(9.2) | 276 280 318 340 374 525<br>(28.7) S (13.4)(13.5)(11.0)(8.3) |

column (2 × 4 cm) of Dowex 50-X2 ($H^+$) (200–400 mesh). The column was washed with water and the nucleoside eluted with 1 M ammonium hydroxide. The eluate was evaporated *in vacuo* to a syrup that was crystallized from methanol: yield 433 mg (84%) softens at 184–186°, dec 210°; $\lambda_{max}^{MeOH}$ 258 nm (ε 15,080); nmr ($d_5$-pyridine–$D_2O$) 4.23 (oct, 2, $J_{5a',5b'}$ = 12 Hz, $J_{4',5a'}$ = 6 Hz, $J_{4',5b'}$ = 6 Hz, $C_{5a'}H$ and $C_{5b'}H$), 4.61 (sx, 1, $J_{3',4'}$ = 3 Hz, $C_{4'}H$), 4.95 (m, 2, $C_{2'}H$ and $C_{3'}H$), 7.26 (d, 1, $J_{1'2'}$ = 4 Hz, $C_{1'}H$), 8.52 (s, 1, $C_2H$ or $C_8H$), 8.66 ppm (s, 1, $C_2H$ or $C_8H$); nmr ($d_6$-$Me_2SO$) 5.78 (m, 2, $C_{2'}OH$ and $C_{3'}OH$) and 7.24 ppm (s, 2, $NH_2$). *Anal.* Calcd for $C_{10}H_{12}ClN_5O_3$: C, 42.03; H, 4.20; Cl, 12.43; N, 24.52. Found: C, 41.82; H, 4.31; Cl, 12.47; N, 24.25.

## Results

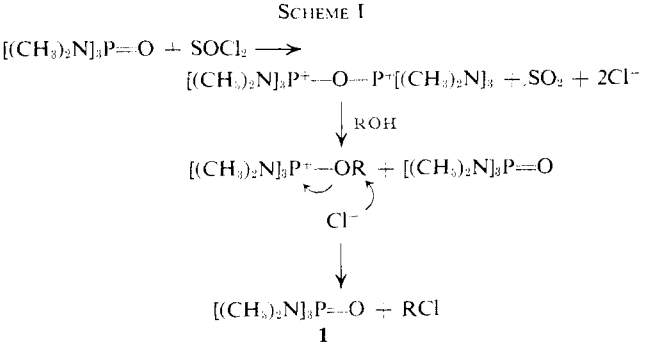
*Properties of the Analogs.* The absorption spectra of adenosylcobalamin and its analogs are presented in Table II. The spectra of several analogs, prepared by different chemical routes, have been published before and are not included (Hogenkamp *et al.*, 1971). The visible and ultraviolet absorption spectra of the cobalamins containing nebularine, L-adenosine, aristeromycin, tubercidin, *ara*-adenosine, and 9-(3′-hydroxypropyl)adenine are very similar to that of adenosylcobalamin. The spectra below 300 nm of the cobalamins containing 3-isoadenosine, inosine, cytidine, and 1-β-D-ribofuranosylbenzimidazole are determined by the spectral properties of the nucleoside, while the spectra of the cobalamins derived from 9-(4′-hydroxybutyl)adenine and 9-(5′,6′-dideoxy-β-D-*ribo*-heptofuranosyl)adenine resemble that of ethylcobalamin with a broad maximum at 509 nm. The spectrum of 3-isoadenosylcobalamin is distinct from the spectra of the other cobalamins by a pronounced γ-band at 350 nm. As in the case of adenosylcobalamin, the addition of acid to aqueous solutions of the analogs causes a spectral shift to a lower wavelength with new maxima at approximately 460 nm. The $pK_a$ values estimated from the midpoint of these spectral changes fall between pH 3 and 4.

All the analogs contain a carbon–cobalt bond and are photolabile; photolysis in the presence of air yields aquocobalamin and presumably a mixture of nucleoside aldehyde and cyclic nucleoside (Hogenkamp, 1964). The cobalamins containing 9-(3′-hydroxypropyl)adenine, 9-(4′-hydroxybutyl)-adenine, 9-(5′,6′-dideoxy-β-D-*ribo*-heptofuranosyl)adenine, and aristeromycin are not decomposed by 0.1 M cyanide in the dark; these observations are in accord with those of Kerwar *et al.* (1970) who also showed that aristeromycylcobalamin is stable in the presence of cyanide. Furthermore even in 1 M potassium cyanide the spectra of these four cobalamins do not undergo significant changes, indicating that the 5,6-dimethylbenzimidazole moiety is not readily displaced by cyanide ion.

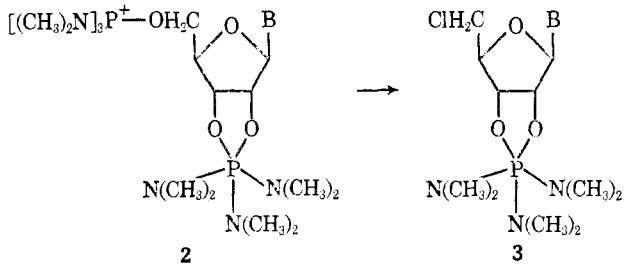
## Discussion

The direct halogenation of primary alcohols with the hexamethylphosphoramide–thionyl chloride reagent probably proceeds *via* an ionic mechanism as shown in Scheme I (Gawne *et al.*, 1969). This mechanism involves the intermediate formation of an alkyloxyphosphonium ion (**1**) which is displaced by chloride ion to yield the desired alkyl halide. Similarly, reaction of the hexamethylphosphoramide–thionyl chloride reagent with ribonucleosides probably involves first reaction with the primary hydroxyl group at carbon 5′ followed by reaction with the secondary hydroxyl groups. However the oxyphosphonium intermediates at either carbon 2′ or 3′ are readily converted to a 2′,3′-cyclic intermediate (**2**) as a result of attack by the cis-vicinal hydroxyl group and

SCHEME I



thus only the oxyphosphonium ion at carbon 5′ can be displaced by chloride ion. This cyclic intermediate (**3**) decom-



poses to the 5′-deoxy-5′-chloronucleoside only during the work-up of the reaction. Reaction of the 2′-deoxyribonucleosides, which are unable to form a 2′,3′-cyclic intermediate, with the hexamethylphosphoramide thionyl chloride reagent leads to the formation of the corresponding 2′,3′,5′-trideoxy-3′,5′-dichloronucleosides. Reaction of 2′,3′,5′-trideoxy-3′,5′-dichloroadenosine with cob(I)alamin yields a cobalamin (presumably 2′,3′-dideoxy-3′-chloroadenosylcobalamin) which is unstable under the reaction conditions and decomposes to hydroxycobalamin.

Chlorination of the deoxyribonucleosides at C-3′ should occur with inversion of configuration to yield the threo isomers (Verheyden and Moffatt, 1972). The nmr spectra of the three dichloronucleosides are consistent with the threo configuration, indicating that chlorination at C-3′ has indeed taken place with inversion of configuration. In both 2′,3′,5′-trideoxy-3′,5′-dichlorouridine and 3′,5′-dideoxy-3′,5′-dichlorothymidine the C-2a′ and C-2b′ protons show markedly different chemical shifts, while the C-1′ proton appears as a quartet because the coupling constants between $C_{1'}H$ and the two C-2′ protons are quite distinct (*e.g.*, for dideoxydichlorothymidine $J_{1'2a'}$ = 3.7 Hz and $J_{1'2b'}$ = 8 Hz). In contrast, in 3′-substituted thymidine analogs with the erythro configuration the C-2′ protons have very similar chemical shifts and the C-1′ proton appears as a triplet (Verheyden and Moffatt, 1970; Slessor and Tracey, 1973). The nmr spectrum of 2′,3′,5′-trideoxy-3′,5′-dichloroadenosine is also quite different from that of 2′-deoxyadenosine (Slessor and Tracey, 1973). In the spectrum of this chlorinated nucleoside the C-1′ proton appears as a quartet, while in the deoxyadenosine spectrum the $C_{1'}H$ appears as a triplet. Furthermore the introduction of the chlorine atom at C-3′ has resulted in an upfield shift of the C-2b′ protons so that the order of the two C-2′ protons is reversed.

Reaction of *ara*-adenosine with the hexamethylphosphoramide–thionyl chloride reagent leads to the formation of 5′-chloro-5′-deoxy-*ara*-adenosine. Reaction of this chloronucleoside with cob(I)alamin yields *ara*-adenosylcobalamin. The reaction with cob(I)alamin indicates the location of the chlorine atom at carbon 5′, because secondary alkyl halides are unable to react with cob(I)alamin to form a carbon–cobalt bond (Brodie, 1969). The nmr spectra are also consistent with this structure, the coupling constant between $C_{1'}H$ and $C_{2'}H$ is identical with that of *ara*-adenosine ($J_{1',2'}$ = 4 Hz) indicating that no changes at C-2′ have taken place. This selective chlorination of *ara*-adenosine at the 5′ position indicates that the two secondary hydroxyl functions are protected during the reaction. It is probably that oxyphosphonium intermediates are formed at both carbon 2′ and 3′ of the nucleoside. However, subsequent displacement by chloride ion is not possible because the bulky hexamethylphosphonium groups prevent the attack by halide ion.

Reaction of *xylo*-adenosine with the chlorinating reagent yields a new nucleoside which did not react with cob(I)alamin to form a carbon–cobalt bond. A cyclic intermediate, similar to **3** involving carbons 3′ and 5′ is probably formed when *xylo*-adenosine is reacted with the hexamethylphosphoramide–thionyl chloride reagent. This intermediate precludes further reaction at carbons 3′ and 5′ and thus only the oxyphosphonium adduct at C-2′ is susceptible to attack by chloride ion, yielding a 2′-deoxy-2′-chloronucleoside which is unable to react with cob(I)alamin (Brodie, 1969). Because of the scarcity of *xylo*-adenosine, the chlorinated product could not be characterized. Reaction of 4′-thioadenosine, 3′-amino-3′-deoxyadenosine, and guanosine with the hexamethylphosphoramide–thionyl chloride reagent gave several products none of which reacted with cob(I)alamin to yield a cobalamin with a carbon–cobalt bond. Reaction of formycin with the chlorinating reagent gave a new nucleoside, which was strongly fluorescent in ultraviolet light. However, reaction of this nucleoside with cob(I)alamin did not yield the desired formycylcobalamin. Unfortunately most of these nucleosides were only available in small quantities so that the chlorinated intermediates could not be characterized.

The partial synthesis of cobalamin analogs *via* the 5′-deoxy-5′-chloronucleosides is the most convenient method presently available. For instance using this method aristeromycylcobalamin could be prepared from 53 mg of aristeromycin in 70% yield; in comparison using conventional techniques Kerwar *et al.* (1970) starting from 10 mg of the nucleoside obtained 14 mg of a mixture of two cobalamins: aristeromycylcobalamin and probably aristeromycylcobalamin-*e*-carboxylic acid (Morley *et al.*, 1968).

The physical and chemical properties of the cobalamin analogs, in which the adenosyl moiety of the coenzyme is replaced by another nucleoside, are very similar to those of adenosylcobalamin. Like adenosylcobalamin the analogs lack the prominent absorption peak at 361 nm of cyanocobalamin. Furthermore all analogs are photolabile and are converted to aquocobalamin in the presence of light. However, unlike adenosylcobalamin, the analogs containing aristeromycin, 9-(5′,6′-dideoxy-β-D-*ribo*-heptofuranosyl)adenine, 9-(3′-hydroxypropyl)adenine, and 9-(4′-hydroxybutyl)adenine are not decomposed by cyanide ion. These analogs are similar to the simple alkylcobalamins, which are also stable in the presence of cyanide. Evidently the incipient carbanion which would be formed as a result of heterolytic cleavage of the carbon–cobalt bond cannot be stabilized in these analogs. Mild acid hydrolysis of adenosylcobalamin yields aquocobalamin, adenine, and D-*erythro*-2,3-dihydroxpent-4-enal (Hogenkamp, 1964); similar treatment of the four analogs does not cause cleavage of the carbon–cobalt bond. However, under these conditions slow hydrolysis of the propionamide side chain *e* to the corresponding carboxylic acid would be expected.

## Acknowledgment

The author is indebted to the generous donors whose gifts of nucleosides are acknowledged above. The 220-MHz nmr spectra were determined by Mr. M. Miller from the Chemistry Department of the University of Illinois, Urbana, Ill.

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