Intramolecular Free Radical Functionalisation of the Methyl Group of 5′-Deoxyadenosine

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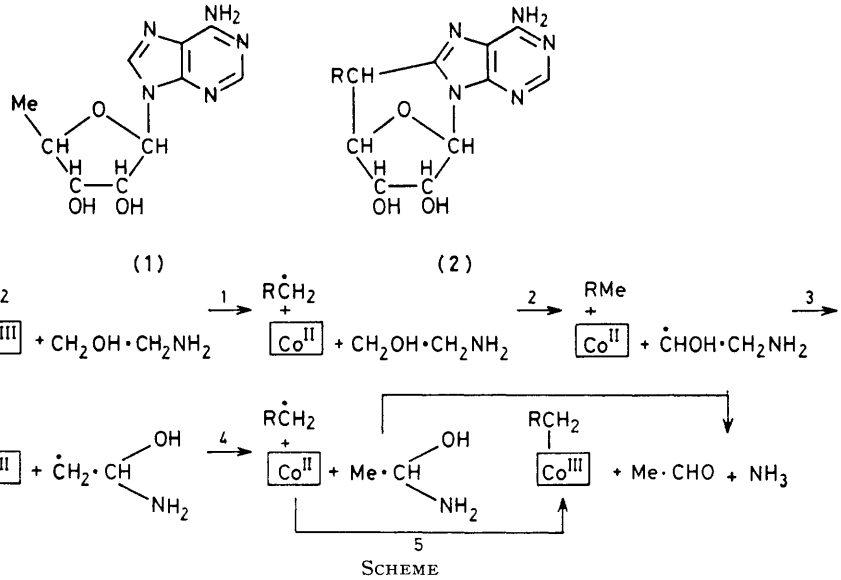
Formation (2 methods) of a free radical at the 8-position of 5′-deoxy-2′,3′-*O*-isopropylideneadenosine causes cyclisation with the 5′-methyl group to give, in yields of up to 80%, 5′-deoxy-2′,3′-*O*-isopropylidene-5′,8-cycloadenosine, identical with the product formed from the 5′-radical by cyclisation with the 8-position. The reactions provide an *in vitro* analogy for the functionalisation of the methyl group of 5′-deoxyadenosine postulated as a step in the mechanism of many enzymic coenzyme B_{12}-controlled rearrangement reactions.

The mechanism of the enzymic B_{12}-catalysed rearrangement reactions is believed by most authors to involve free-radical intermediates initiated by the homolytic fission of the cobalt–carbon bond of the coenzyme [1,2] (step 1 in the Scheme, showing the catalysis in the ethanolamine ammonia–lyase system; for simplicity, possible protonation of substrate is excluded). Within the special constraints of the enzyme this is caused by the approach of the substrate to the reactive centre and presumably occurs to relieve strain. The next step (2 in the Scheme, which may be considered as the first reaction in the catalytic cycle) is believed to be an abstraction of hydrogen from the substrate by the 5′-deoxyadenosyl radical, which is thereby converted into 5′-deoxyadenosine (1).† After rearrangement of the substrate radical (step 3), the product radical reverses the process by abstracting hydrogen from 5′-deoxyadenosine (step 4), reforming the 5′-deoxyadenosyl radical which is available to continue the catalysis (step 1) or to reform the coenzyme (step 5).

Of the steps postulated in the Scheme, no parallel existed for the facile chemical or microbiological functionalisation of the 5′-methyl group of 5′-deoxyadenosine (step 4 in the Scheme). However, in earlier studies on model systems, we have provided analogies for steps 1,[3a] 2,[3b] and 3 in the Scheme. Schrauzer *et al.*[4] have claimed to have detected trace amounts of the B_{12} coenzyme when 5′-deoxyadenosine and cob(II)alamin were allowed to react in the presence of vanadium(III) chloride but no details of these experiments are available.

Early work on the chemical reactions of the B_{12} coenzyme [5,6] had demonstrated that anaerobic photolysis gave 5′-deoxy-5′,8-cycloadenosine (2; R = H) by homolytic fission of the cobalt–carbon bond, and reaction of the 5′-deoxyadenosyl radical with the adjacent 8-position of the purine ring. More recently it has been shown [7] that a similar cyclisation occurs when 5′-deoxy-5′-phenylthio-2′,3′-*O*-isopropylideneadenosine is irradiated in the presence of triethyl phosphite, and again when N^6-dibenzoyl-5′-deoxy-5′-iodo-2′,3′-*O*-isopropylideneadenosine [8] is treated with tri-n-butyltin hydride in the presence of an initiator.[9] Accordingly we surmised that a radical at the 8-position of 5′-deoxyadenosine might also yield (2; R = H) by intramolecular cyclisation with the 5′-methyl group,[10] and indeed Matsuda and his co-workers [11] have reported that 8-phenylthio-2′,3′-*O*-isopropylideneadenosine (3; R = OH), when photolysed in presence of radical initiators, yields both 5′-enantio-

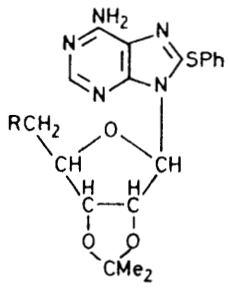


Scheme

† The conformation of the adenosines prepared during these studies will be discussed in a forthcoming paper.

mers of 2′,3′-*O*-isopropylidene-5′,8-cycloadenosine (4; R = OH).

Our projected cyclisation thus required the preparation



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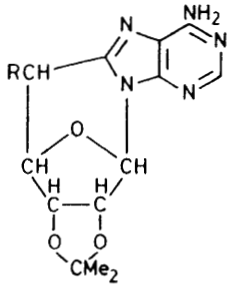

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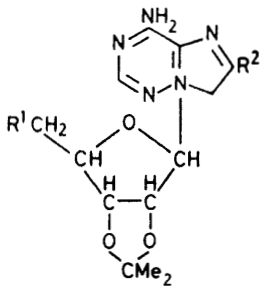
of 5′-deoxy-8-phenylthio-2′,3′-*O*-isopropylideneadenosine (3; R = H), which required 5′-deoxy-2′,3′-*O*-isopropylideneadenosine (5; $R^1 = R^2 = H$) as the key intermediate. Several non-enzymic syntheses of this substance or the parent 5′-deoxyadenosine have been reported. The 5′-methyl group has been obtained by hydrogenolysis of either 5′-deoxy-5′-iodo-[12a,b] or 5′-deoxy-5′-thio [1,12c] derivatives, but the overall yields from adenosine did not exceed 20%. We therefore investigated alternative methods including the attempted dechlorination of 5′-deoxy-5′-chloro-2′,3′-*O*-isopropylideneadenosine (5; $R^1 = Cl$, $R^2 = H$). Hydrogenolysis over palladium–charcoal or reaction with sodium borohydride or lithium aluminium hydride in tetrahydrofuran at room temperature failed to remove the chlorine and lithium aluminium hydride in refluxing tetrahydrofuran gave a complex mixture of products. Sodium hydride in dimethylformamide at 90 °C gave a product which was possibly the quaternary chloride of 2′,3′-*O*-isopropylidene-3,5′-cycloadenosine. However we have also found that reaction of the 5′-chloro-derivative (5; $R^1 = Cl$, $R^2 = H$) with thiophenol in refluxing



(5)

methanol in the presence of an excess of sodium methoxide gave the 5′-phenylthio-derivative (5; $R^1 = SPh$, $R^2 = H$) in 70% yield, and treatment of this with Raney nickel at 100 °C then gave *ca.* 80% of 5′-deoxy-2′,3′-*O*-isopropylideneadenosine (5; $R^1 = R^2 = H$). This synthesis is therefore a considerable improvement over earlier preparations.

Bromination of (5; $R^1 = R^2 = H$) in a manner analogous to the bromination of 5′-*O*-substituted adenosines,[13,14] gave the 8-bromo-derivative (5; $R^1 = H$, $R^2 = Br$) in quantitative yield. Further reaction of the bromo-compound with methanolic thiophenol in the presence of sodium methoxide then gave 60% of the required 5′-deoxy-8-phenylthio-2′,3′-*O*-isopropylideneadenosine (3; R = H) together with 30% of the corresponding 8-methoxy-compound (5; $R^1 = H$, $R^2 =$ OMe). Although 8-bromo-5′-deoxyadenosine with refluxing methanolic sodium methoxide is converted into the 8-methoxy-derivative only in moderate yield after 18 h,[15] we have shown that with (5; $R^1 = H$, $R^2 = Br$), the substitution is quantitative after 1 h at 100 °C. At higher temperatures, the 8-hydroxy-derivative (or its lactam tautomer) is also produced. However the 8-phenylthio-2′,3′-*O*-isopropylidene derivatives of both adenosine and 5′-deoxyadenosine with an excess of methanolic sodium methoxide at *ca.* 55 °C also produce high yields of the corresponding 8-methoxy-compounds and consequently the phenylthio compounds could be intermediates in the observed formation of the 8-methoxy-compounds during reaction of the 8-bromo-compounds with methanolic PhSNa.

5′-Deoxy-8-phenylthio-2′,3′-*O*-isopropylideneadenosine was dissolved in acetonitrile and, after addition of a little t-butyl hydroperoxide as radical initiator, was purged with argon and irradiated for several hours with u.v. light at 250—350 nm (*cf.* ref. 10). The product was shown to be (4; R = H) by direct comparison with material formed by the anaerobic irradiation of the 2′,3′-*O*-isopropylidene derivative of the B_{12} coenzyme.[5,6] None of the corresponding 7,8-dihydro-derivative of the 5′-deoxy-5′,8-cycloadenosine (*cf.* ref. 9) was detected in the products. The ^1H n.m.r. spectrum of the 5′-deoxy-2′,3′-*O*-isopropylidene-5′,8-cycloadenosine showed no coupling between the 1′- and 2′-protons and a model showed a dihedral angle of *ca.* 90° between these protons. The 2′- and 3′-protons appeared to be magnetically equivalent and appeared as a singlet and no coupling was observed between the 3′- and 4′-protons, which also showed a 90° dihedral angle. The angle between the 4′- and $5'_a$-proton was *ca.* 80°, causing the observed coupling of 1 Hz, whereas between the 4′- and $5'_b$-protons, the angle was *ca.* 30°, causing a coupling of 5.5 Hz. A large coupling 17 Hz, was observed between the $5'_a$- and $5'_b$-protons.

The 8-radical of 5′-deoxy-2′,3′-*O*-isopropylidene adenosine was also generated from the 8-bromo-derivative (5; $R^1 = H$, $R^2 = Br$) by the general method due to Bowles, Hudson, and Jackson.[16] Thus the compound was irradiated under anaerobic conditions with tri-n-propylsilane in the presence of di-t-butyl peroxide. After several hours over half of the starting material was converted into two products which were separated. The less polar product was shown to be 5′-deoxy-2′,3′-*O*-isopropylideneadenosine (5; $R^1 = R^2 = H$), presumably formed by abstraction of a hydrogen atom from the solvent, and and the more polar product (30%) was 5′-deoxy-2′,3′-*O*-isopropylidene-5′,8-cycloadenosine (4; R = H).

Hence the methyl group of 5′-deoxyadenosine is capable of attack by neighbouring free radicals, and the experiments described leading to formation of 5′-deoxy-5′,8-cycloadenosine derivatives provide an *in vitro* analogy for the B_{12}-catalysed rearrangement reactions.

EXPERIMENTAL

U.v. spectra were recorded with a Pye Unicam SP8000 instrument. ^{1}H N.m.r. spectra were obtained using Perkin-Elmer R 32 (90 MHz) and R 12 (60 MHz) continuous-wave machines with an internal standard of 1% tetramethylsilane. Photochemical reactions were carried out in Pyrex vessels inside an 800 W Rayonet preparative reactor (RPR 208) fitted with four 350 nm and four 300 nm tubes. All reagents and solvents were of the highest commercially available quality and liquid reagents were distilled and dried before use. Raney nickel [17] was freshly prepared for each reduction.

5′-Chloro-5′-deoxy-2′,3′-O-isopropylideneadenosine (5; R^1 = Cl, R^2 = H).—5′-Chloro-5′-deoxyadenosine [18] (1 g, 3.5 mmol) was dissolved in dry acetone (35 ml) containing fused $ZnCl_2$ (3 g, 22 mmol) and the solution was heated under reflux for 6 h and then slowly cooled. The product was evaporated (to *ca.* 10 ml) and the residue was poured into a solution of baryta [9 g $Ba(OH)_2 \cdot 8H_2O$ in 70 ml H_2O] at 40 °C with stirring. The product was cooled to 20 °C and carbon dioxide was bubbled through the solution to pH 6.5. The precipitated carbonates were separated and washed with boiling water (2 × 30 ml) and boiling methanol (2 × 30 ml). The combined extracts were evaporated to *ca.* 50 ml and then extracted with chloroform (3 × 50 ml), and the combined chloroform extracts were dried. Removal of the solvent gave the product (0.82 g, 75%), identical with that obtained by chlorination of 2′,3′-*O*-isopropylideneadenosine with thionyl chloride and pyridine,[19,20] $\lambda_{max.}$ (H_2O, pH 7), 260 nm (ε 14 500); δ[$(CD_3)_2SO$] 8.37 (1 H, s, 8-H of purine), 8.22 (1 H, s, 2-H of purine), 7.36br (2 H, s, 6-NH_2), 6.27 (1 H, d, *J* 2 Hz, 1′-H of ribose), 5.52 (1 H, dd, *J* 2 and 6 Hz, 2′-H of ribose), 5.10 (1 H, dd, *J* 6 and 3 Hz, 3′-H of ribose), 4.34 (1 H, td, *J* 3 and 8 Hz, 4′-H or ribose), 3.80 (2 H, dd, *J* 8 and 3 Hz, 5′-H_2 of ribose), and 1.53 and 1.32 (both 3 H, s, acetonide).

5′-Deoxy-5′-phenylthio-2′,3′-O-isopropylideneadenosine (5; R^1 = SPh, R^2 = H).—The foregoing 5′-chloro-derivative (2 g, 6.1 mmol) was dissolved in dry methanol (40 ml) and thiophenol (5 ml, 45 mmol) was added, followed by sodium (0.9 g, 39 mmol). The dark red solution was heated under reflux for 4 h, after which t.l.c. on silica (10 : 1 v/v $CHCl_3$–MeOH for elution) showed only a trace of starting material. Ice and dilute hydrochloric acid (10 ml; 2M) were added slowly to the cooled stirred solution and the methanol then removed *in vacuo*. The aqueous residue was extracted with chloroform (5 × 50 ml) and the combined extracts were washed with water (2 × 5 ml). The chloroform layer was treated with activated charcoal (1 g) and magnesium sulphate (6 g), filtered, and the solvent was removed. The resulting brown sticky solid was dissolved in chloroform (3 ml) and chromatographed on silica (35 × 3 cm) using chloroform to elute the excess of thiophenol and then chloroform–methanol (6 : 1 v/v) to yield, after removal of solvent, the product as a pale fawn powder (1.72 g, 70%) (Found: M^+, 399. $C_{19}H_{21}N_5O_3S$ requires *M*, 399); $\lambda_{max.}$ (H_2O at pH 7) 255 nm (ε 15 900); δ 8.21 (1 H, s, 2-H of purine), 7.76 (1 H, s, 8-H of purine), 7.18 (5 H, m, benzenoid H), 6.02br (2 H, s, 6-NH_2), 5.59 (1 H, d, *J* 2 Hz, 1′-H of ribose), 5.51 (1 H, dd, *J* 6 Hz and 2 Hz, 2′-H of ribose), 5.08 (1 H, dd, *J* 3 Hz and 6 Hz, 3′-H of ribose), 4.30 (1 H, td, *J* 7 Hz and 3 Hz, 4′-H of ribose), 3.25 (2 H, d, *J* 7 Hz, 5′-H_2 of ribose), and 1.65 and 1.46 (each 3 H, s, acetonide).

5′-Deoxy-2′,3′-O-isopropylideneadenosine (5; R^1 = R^2 = H).—The foregoing 5′-phenylthio-derivative (800 mg, 2 mmol) was dissolved in dry methanol (20 ml) and Raney nickel (6 ml in dry methanol) was added. The suspension was warmed to 50 °C, transferred to a pressure hydrogenator, and heated at 110 °C for 90 min with occasional shaking. After cooling, the contents were removed, and the nickel was separated and washed with hot ethanol. The combined filtrates were evaporated (to *ca.* 2 ml) and the residue applied to four silica gel plates (20 × 20 cm × 1.2 mm) and developed with $CHCl_3$–MeOH (10 : 1 v/v). The more polar band was removed, suspended in $CHCl_3$–MeOH (3 : 1 v/v), filtered, and the solvent was removed leaving the product as a colourless microcrystalline solid (510 mg, 88%), m.p. 206—208 °C, λ(H_2O at pH 7) 259 nm (ε 14 000). Found: M^+, 291. Calc. for $C_{13}H_{17}N_5O_3$: *M*, 291); δ 8.33 (1 H, s, 2-H of purine), 7.87 (1 H, s, 8-H of purine), 6.23br (2 H, s, N^6-H_2), 6.00 (1 H, d, *J* 3 Hz, 1′-H of ribose), 5.48 (1 H, dd, *J* 7 Hz and 3 Hz, 2′-H of ribose), 4.71 (1 H, dd, *J* 4 Hz and 7 Hz, 3′-H of ribose), 4.3 (1 H, qd, *J* 7 Hz and 4 Hz, 4′-H of ribose), 1.49 and 1.28 (6 H, s, acetonide), and 1.26 (3 H, d, *J* 7 Hz, 5′-H of ribose).

8-Bromo-5′-deoxy-2′,3′-O-isopropylideneadenosine (5; R^1 = H, R^2 = Br).—The foregoing 5′-deoxy-derivative (220 mg, 0.76 mmol) in ethanol (5 ml) was mixed with acetate buffer [from acetic acid (2 g), sodium acetate (6 g), and water (40 ml)]. Bromine (0.6 g, 3.75 mmol) was added in drops over 5 min to the stirred solution, and after 2 h the excess of bromine was removed with sodium metabisulphite. After addition of more water (50 ml) the solution was adjusted to pH 8 and then extracted with chloroform (3 × 15 ml) and backwashed with water. The chloroform extracts were dried and evaporated to yield the *bromo-derivative* as a pale yellow amorphous solid (180 mg, 65%) (Found: M^+, 370. $C_{13}H_{16}BrN_5O_3$ requires *M*, 370), m.p. 139 °C (decomp.); λ(H_2O, pH 7) 266 nm (ε 17 500); δ 8.26 (1 H, s, 2-H of purine), 6.15 (1 H, d, *J* 2 Hz, 1′-H of ribose), 5.80 (1 H, dd, *J* 7 and 2 Hz, 2′-H of ribose), 4.97 (1 H, dd, *J* 5 and 7 Hz, 3′-H of ribose), 4.40 (1 H, m, 4′-H of ribose), 1.73 and 1.51 (each 3 H, s, acetonide), and 1.43 (3 H, d, *J* 6 Hz, 5′-CH_3).

5′-Deoxy-8-phenylthio- (5; R^1 = H, R^2 = SPh) *and 5′-Deoxy-8-methoxy-2′,3′-O-isopropylideneadenosine* (5; R^1 = H, R^2 = OMe).—The above 8-bromo-derivative (150 mg, 0.4 mmol) was dissolved in dry methanol (20 ml), and thiophenol (1 g, 9.1 mmol) was added. A slow stream of nitrogen was passed through the solution while sodium (0.25 g, 10.9 mmol) was added, causing the reaction mixture to become dark red. The solution was warmed to 55 °C for 2 h when t.l.c. showed 2 bands (SiO_2 plates with 5 : 1 v/v $CHCl_3$–MeOH; R_F 0.63 and 0.57). Water (5 ml) was added and the solution was neutralised with 1M-hydrochloric acid. Methanol was removed *in vacuo* and the residual aqueous solution extracted with chloroform (3 × 20 ml). After removal of solvent from the combined extracts, the excess of thiophenol was partly removed by heating at 40 °C and 0.3 Torr for 2 h and the residual yellow oil was dissolved in chloroform (3 ml) and applied to silica gel plates (20 × 20 × 1.2 mm) using chloroform–methanol (10 : 1 v/v) for elution. The less polar band was removed and suspended

in chloroform–methanol (3 : 1 v/v), the silica was separated, and the solvent was evaporated off to yield 5′-*deoxy*-8-*phenylthio*-2′,3′-O-*isopropylideneadenosine* as an amorphous pale fawn powder (88 mg, 55%) (Found: M^+, 399. $C_{19}H_{21}N_5O_3S$ requires M, 399). $\lambda_{max.}$ [H_2O–MeOH (20 : 1 v/v) at pH 7] 282 nm (ε 17 500); δ 8.08 (1 H, s, 2-H of purine), 7.15 (5 H, m, phenyl), 6.16 (1 H, d, J 3 Hz, 1′-H of ribose), 5.55 (1 H, dd, J 6 and 3 Hz, 2′-H of ribose), 4.78 (1 H, dd, J 4 and 6 Hz, 3′-H of ribose), 4.15 (1 H, qd, J 6 and 4 Hz, 4′-H of ribose), 1.55 and 1.34 (each 3 H, s, acetonide), and 1.32 (3 H, d, J 6 Hz, 5′-methyl).

The more polar band was removed from the plates and suspended in chloroform–methanol (3 : 1 v/v). Removal of the silica and evaporation of the solvent gave 5′-*deoxy*-8-*methoxy*-2′,3′-O-*isopropylideneadenosine* as a pale yellow amorphous solid (29 mg, 22%) which formed off-white crystals (from methanol), m.p. 185—186 °C (Found: M^+, 321. $C_{14}H_{19}N_5O_4$ requires M, 321). $\lambda_{max.}$ [H_2O–MeOH, 20 : 1 (v/v), pH 7] 258 nm (ε 17 200); δ 8.10 (1 H, s, 2-H of purine), 6.01 (1 H, d, J 3 Hz, 1′-H of ribose), 5.59 (1 H, dd, J 6 and 3 Hz, 2′-H of ribose), 4.80 (1 H, dd, J = 4 and 6 Hz, 3′-H of ribose), 4.29 (1 H, qd, J 6 and 4 Hz, 4′-H of ribose), 4.18 (3 H, s, OCH_3), 1.70 and 1.48 (each 3 H, s, acetonide), and 1.43 (3 H, d, J 6 Hz, 5′-methyl).

5′-*Deoxy*-2′,3-O-*isopropylidene*-5′,8-*cycloadenosine* (4; R = H).—(*i*) 5′-Deoxy-8-phenylthio-2′,3′-*O*-isopropylideneadenosine (above, 80 mg, 0.2 mmol) was dissolved in acetonitrile (80 ml) and t-butyl hydroperoxide (4 ml; 70%) was added. The solution was purged with argon and sealed in a Pyrex flask which was irradiated with u.v. light (250—350 nm, 800 W) at *ca.* 20 cm distance. After 85 h at 35 °C, t.l.c. on silica in 10 : 1 (v/v) $CHCl_3$–MeOH revealed one major band (R_F 0.42) and no starting material. The solvent was removed *in vacuo* from the major fraction and the resulting colourless oil was dissolved in $CHCl_3$ (3 ml) and applied to three silica plates (20 × 20 cm × 1.2 mm) using 25.1 (v/v) $CHCl_3$–MeOH for elution. The lower halves of the plates were removed and suspended in 3 : 1 (v/v) $CHCl_3$–EtOH and stirred for 10 min. After filtration the filtrate was reduced to *ca.* 3 ml and then reapplied to 3 plates using 8 : 1 (v/v) $CHCl_3$–MeOH for elution. The main band, R_F 0.45 was removed, suspended in 3 : 1 (v/v) $CHCl_3$–EtOH and filtered. Removal of the solvent from the filtrate gave the cyclonucleoside as an off-white solid (38 mg, 66%), m.p. 233—234 °C (Found: M^+, 289. Calc. for $C_{13}H_{15}N_5O_3$: M 289); $\lambda_{max.}$ (H_2O, pH 7) 263 nm (ε 17 500); δ 8.03 (1 H, s, 2-H of purine), 6.08 (1 H, s, 1′-H of ribose), 4.69 [1 H, dd, J (4′,5′H_b) 5.5, J (4′,5′H_a) 1 Hz, 4′-H of sugar], 4.52 (2 H, s, 2′-H and 3′-H of ribose), 3.41 (1 H, dd, 5′$_b$-H of ribose, $J_{5'a,5'b}$ 17, $J_{4',5'b}$ 5.5 Hz), 2.83 (1 H, dd, 5′$_a$-H of ribose, $J_{5'a,5'b}$ 17, $J_{4',5'a}$ 1 Hz), and 1.44 and 1.20 (each 3 H, s, acetonide).

(ii) 8-Bromo-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (100 mg) was dissolved in acetonitrile (80 ml) and di-t-butyl peroxide (1.5 ml) was added. The solution was purged with argon for 10 min and tri-n-propylsilane (1 ml) was added. The flask was then irradiated with an 800 W u.v. lamp for 30 h at 35 °C when t.l.c. revealed that all of the starting product had reacted and that two products had been formed. The major, more polar, product [R_F on silica using 8 : 1 (v/v) chloroform–methanol for elution, 0.45] was separated, suspended in 3 : 1 (v/v) chloroform–methanol and the silica removed. Evaporation of the solvent gave the product (22 mg; 30%) which proved to be identical (R_F, u.v. and n.m.r. spectra) with the product from the previous experiment. The minor, less polar, product was isolated as crystals by a similar method (8 mg, 10%) and proved to be 5′-deoxy-2′,3′-O-isopropylideneadenosine, identical with that described above.

(iii) Hydroxocobalamin (500 mg) was dissolved in water (20 ml) and cobalt(II) acetate (5 mg) was added. When dissolution was complete, methanol (10 ml) was added and the solution was deoxygenated by passing a stream of nitrogen through it. After 15 min, sodium borohydride (200 mg) was added causing the solution to change from red through brown to green. The flask was protected from light and 5′-chloro-2′,3′-*O*-isopropylideneadenosine (220 mg) was added. After a few min the colour reverted to red, when it was washed with chloroform (2 × 50 ml) and then extracted with phenol–chloroform (1 : 1 v/v; 3 × 20 ml). The phenolic solution was washed with water (2 × 10 ml) and then extracted into water after dilution with chloroform (to 450 ml). The aqueous extracts were combined and washed with chloroform (20 ml) and then ether (2 × 50 ml). The solvents were removed *in vacuo* and examination of the residue (u.v. spectrum and t.l.c.) showed that no hydroxocobalamin remained. The isopropylideneadenosylcobalamin was dissolved in water (150 ml), and the solution was purged with argon for 10 min and then exposed to light from a 150 W tungsten lamp (*ca.* 10 cm distance) with a reduced stream of argon for 2 h when the solution became brown. The photolysis was continued for another 30 min, and then when the solution was exposed to air it became red. The aqueous solution was extracted with chloroform (3 × 20 ml) and the organic solvent was removed from the dried extract. T.l.c. of the colourless residue showed traces of phenol which were removed on three silica gel plates (20 × 20 cm × 1.2 mm) using chloroform–methanol (10 : 1 v/v) for elution. The main band was removed, suspended in chloroform–methanol (3 : 1 v/v), and the silica was removed. Evaporation of the solvent gave a crystalline product (70 mg, 75%), m.p. 233—235 °C, identical (u.v., n.m.r.) with the product obtained in the previous experiment.

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