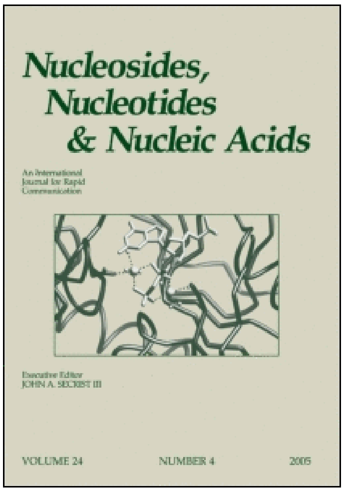

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Didier Betbeder[a]; David W. Hutchinson[a]
[a] Chemistry Department, Warwick University, Coventry, UK

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THE ENZYMATIC SYNTHESIS OF IMIDAZOLE DEOXYNUCLEOSIDES: 1-β-D-2'-DEOXYRIBOFURANOSYL-5-AMINOIMIDAZOLE-4-CARBOXAMIDE AND 1-β-D-2'-DEOXYRIBOFURANOSYLBENZIMIDAZOLE

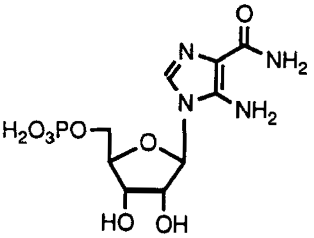
Didier Betbeder and David W Hutchinson*
Chemistry Department, Warwick University, Coventry, CV4 7AL, UK

Abstract

Nucleoside N-deoxyribosyl transferases (EC 2.4.2.6) from *Lactobacillus leichmanii* will catalyse the transfer of 2-deoxyribose from thymidine or 2'-deoxycytidine to 5-aminoimidazole-4-carboxamide or benzimidazole. 1-ß-D-2'-Deoxyribofuranosyl-5-aminoimidazole-4-carboxamide and 1-ß-D-2'-deoxyribofuranosyl-benzimidazole, respectively, are the only products which are formed.

INTRODUCTION

Nucleosides derived from imidazoles and triazoles can have important biological properties. The 5'-monophosphate of 1-ß-D-ribofuranosyl-5-aminoimidazole-4-carboxamide (1) is an intermediate in the biosynthesis of purine nucleotides[1] and the 5'-monophosphate of 1-ß-D-ribofuranosyl-5-aminoimidazole (AIR) is a precursor of the pyrimidine residue of thiamine[2]. The ribonucleoside of 1*H*-1,2,4-triazole-3-carboxamide (2) (ribavirin) is a broad spectrum antiviral agent[3] and 1-ß-D-2'-deoxyribofuranosyl-1,2,3-triazole-4-carboxamide has also been reported to have antiviral activity[4]. Furthermore, 1-ß-D-2'-deoxyribofuranosyl-benzimidazole (3) has been proposed[5] as a nucleoside analogue which might be used for studying stacking interactions in DNA. The benzimi-



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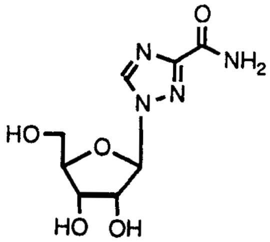

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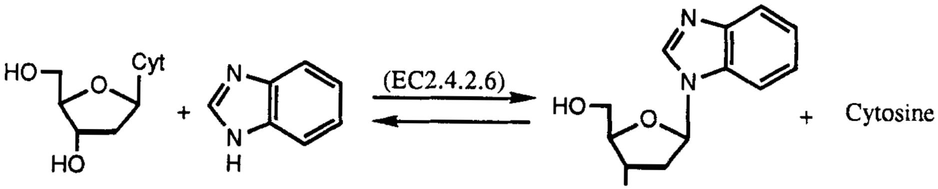

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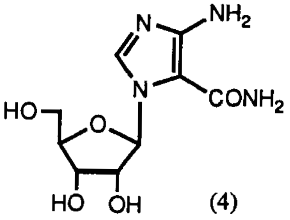

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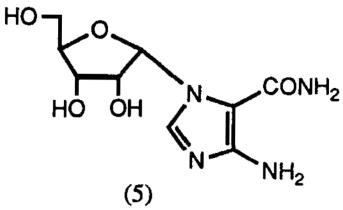

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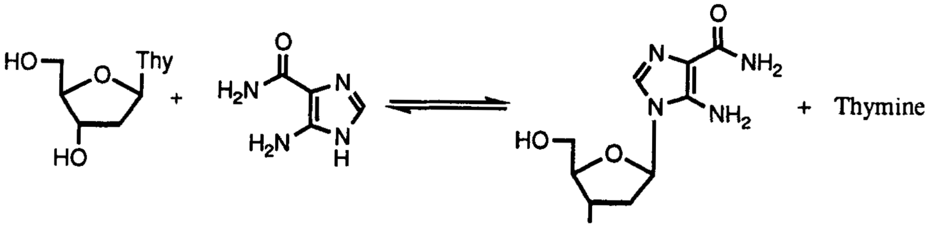


(6)

dazole moiety lacks hydrogen-bonding groups in the benzo-ring and if benzimidazole were to be incorporated into DNA, helical-stabilisation by hydrogen bonding would be impossible. Thus, charge-charge interactions within the DNA chain would become important for the stabilisation of the helix.

A number of chemical syntheses of ribofuranosyl imidazoles have been published[6,7]. However, little has been published on the chemical synthesis of 2'-deoxyribofuranosyl derivatives of imidazoles and triazoles. A mixture of α- and β-anomers of 1-D-2'-deoxyribofuranosyl-4-aminoimidazole-5-carboxamide (4,5) have been obtained "via the fusion procedure"[8]. Low yields of complex mixtures of α- and β-anomers of (N-1) and (N-3) glycosylated products have been reported from reactions between the ethyl ester of 5-aminoimidazole-5-carboxylic acid and 2-deoxyglycosyl chlorides[9]. Compound (3) has been prepared[5] by a multistep procedure from 2-chlorobenzimidazole and 1-*O*-acetyl- 2,3,5-tri-*O*-benzoylribofuranose by Vorbrüggen's procedure[10]. There are no reports of the synthesis of compound (6).

The use of biocatalysts to synthesise nucleosides offers considerable advantage over conventional chemical procedures as precise stereo- and regiochemical control is possible without the use of protecting groups on reactive residues. This might be important in the syntheses of nucleosides derived from imidazoles and related heterocycles which contain several reactive residues. However, the majority of the studies reported so far[11-13] on the enzymatic synthesis of nucleosides have used purines or pyrimidines as glycosyl receptors and there has been little mention of the enzymatic synthesis of nucleosides of heterocycles such as imidazoles or triazoles. Using uridine and purine nucleoside phosphorylases the transfer of the ribose residue from uridine to 1*H*-1,2,4-triazole-3-carboxamide to produce ribavirin (2) has been achieved[14]. A mixture of β-(N-1) and β-(N-3) 2'-deoxynucleosides were obtained from 2'-deoxyuridine and ethyl 5-aminoimidazole-4-carboxylate in a reaction catalysed by auxotroph thymine-dependent *Escherichia coli*[9]. The transfer of ^{14}C-labelled 2-deoxyribose from thymidine to 5-aminoimidazole-4-carboxamide and benzimidazole on a submicromolar scale has been described but no products were isolated and no structural verification of the reacton products was given[15]. Compound (6) has also been synthesised chemically in low yield by a multistage preparation[16]. We now report full experimental details for the enzymatic syntheses of both compound (3) and 1-β-D-2'-deoxyribofuranosyl-5-aminoimidazole-4-carboxamide (6) together with their structural verification by NMR techniques. Our rapid synthetic route should be applicable to the synthesis of deoxynucleosides of other imidazoles and related heterocycles and the simplicity of the experimental procedure may be valuable in the case of labile or reactive heterocycles. For example, compound (6) at room temperature in buffer (sodium citrate, pH 6.0) gives purple products after a few days.

MATERIALS AND METHODS

2'-Deoxycytidine, thymidine and benzimidazole were commercially available from Aldrich Chemical Company Ltd. 5-Aminoimidazole-4-carboxamide was obtained from Sigma Chemical Co Ltd. The crude mixture of N-deoxyribosyl transferases was prepared from *Lactobacillus leichmanii* as previously described[11].

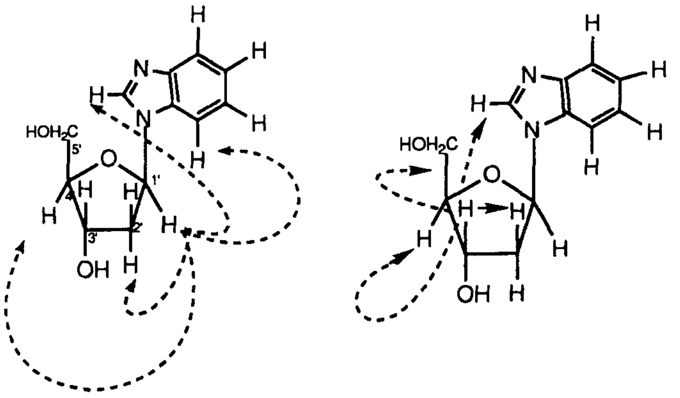
^1H NMR spectra were run at 400 MHz and proton decoupled ^{13}C NMR spectra at 100.62 MHz in d^4-methanol solution. Chemical shifts are given in ppm relative to TMS as internal standard. Nuclear Overhauser enhancement experiments were carried out on solutions of (3) and (6) in d^4-methanol using a preirradiation time of 2 sec and a composite 90° observed pulse to reduce the effects of direct coupling.

High resolution ammonia chemical ionisation (CI) and fast atom bombardment (FAB) mass spectra were recorded on a Kratos MS80 spectrometer.

Deoxyribosyl transfer:

Synthesis of 9-β-D-2'-deoxyribofuranosylbenzimidazole (3) - To benzimidazole (0.338 mmole) and 2'-deoxycytidine (1.355 mmole) in citrate buffer (25 ml, 0.25 mM, pH 6.0) and 0.05% sodium azide were added 24 mg (2.7 U) of crude protein extract at 40°C. The reaction was followed by HPLC (Tecknopak 10 C18 column, elution with a gradient of 15-30% methanol/water). The reaction took 6 days to reach its equilibrium after which time the solution was freeze dried and chromatographed on a silica column, eluted with 10% methanol/dichloromethane. The first compound to appear was unchanged benzimidazole while 1-β-D-2'-deoxyribofuranosylbenzimidazole was in the second fraction to give 33.5 mg (46%) as an oily compound which crystallised slowly at 4°C, running as a single spot on silica TLC R_f = 0.34 ((CH_2Cl_2/EtOH 4/1, v/v). Ammonia CI mass spectrum: $(M + H)^+$ m/z = 235.1056, $C_{12}H_{15}N_2O_3$ requires m/z = 235.1082.

^1H NMR: δ: 8.48 (1Hs, H-8); 7.67 (2Hm, H-3 + H-6); 7.33 (2Hm, H-1 + H-2); 6.44 (1Ht, $J_{1'-2'a}$ = 7.20 Hz, $J_{1'-2'b}$ = 6.24 Hz, H-1'); 4.57 (1Hm, $J_{2'a-3'}$ = 6.85 Hz, $J_{2'b-3'}$ = 3.52 Hz, $J_{3'-4'}$ = 3.89



(a) (b)

FIGURE 1: Sites of nuclear Overhauser enhancements in the ^{1}H NMR spectrum of (3) caused by saturation of the signal due to H-1' (**a**) or H-3' (**b**).

Hz, H-3'); 4.06 (1Hm, $J_{4'-5'a} = J_{4'-5'b}$ = 3.80 Hz, H-4'); 3.78 (2Hm, H-5'a + H-5'b); 2.68 (1Hm, H-2'a); 2.50 (1Hm, H2'b).

^{13}C NMR: δ: 41.32; 63.04; 72.32; 86.41; 88.89; 112.70; 120.22; 123.92; 124.51; 144.36.

Nuclear Overhauser enhancement experiments (Figures 1a and 1b) - Signal enhancements (%) caused by saturation of the signal at 6.44 ppm (H-1'):H-8(3.8); H-2'b(5.4); H-4'(2.2); H-4(1.1). Signal enhancements (%) caused by saturation of the signal at 4.57 ppm (H-3'):H-2'a(5.5); H-4'(1.6); H-5'ab(5.5).

Synthesis of 1-β-D-2'-deoxyribofuranosyl-5-aminoimidazole-4-carboxamide (6) - 5-Aminoimidazole-4-carboxamide (40 mg, 0.32 mmole) and thymidine (240 mg, 1 mmole) were added to a mixture of crude deoxyribosyl transferases (3 ml, 15 mg protein, 6.7 U) in citrate buffer (100 ml, 0.25 mM, pH 6). The reaction reached equilibrium after 6 days as determined by reverse-phase HPLC (Technopak-10 C18 column, elution with a gradient of 15-30% methanol/water). The reaction mixture was lyophilised and the major product purified by flash chromatography on silica (elution with CH_2Cl_2/MeOH 4/1, v/v) to give 65 mg (80%) of (4) as a single compound with an R_f = 0.25 on silica

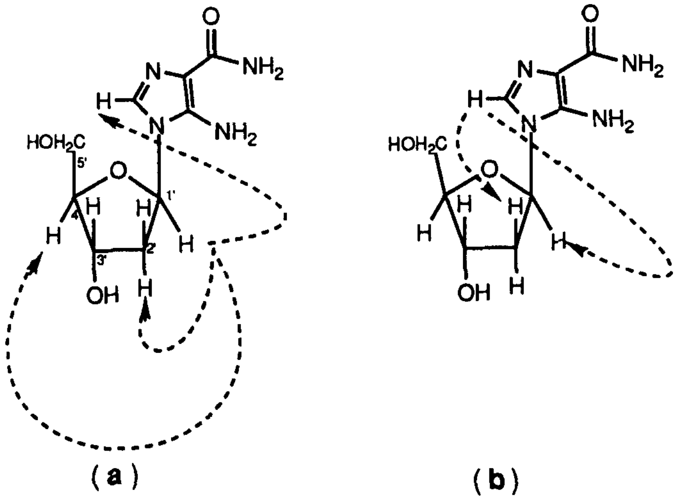


FIGURE 2: Sites of nuclear Overhauser enhancements in the ^{1}H NMR spectrum of (6) caused by saturation of the signal due to H-1' (**a**) or H-2 (**b**).

TLC (CH_2Cl_2/MeOH 4/1, v/v). FAB Mass spectrum (glycerol matrix): $(M + H)^+$ m/z = 243, $(M + Na)^+$ m/z = 265.

^{1}H NMR (CD_3OD): δ: 7.40 (1Hs, H-2), 6.03 (1Hdd, $J_{1'-2'a}$ = 8.00 Hz, $J_{1'-2'b}$ = 6.00 Hz, H-1'), 4.53 (1Hm, $J_{3'-2'a}$ = 6.38 Hz, $J_{3'-2'b}$ = 3.00 Hz, $J_{3'-4'}$ = 3.00 Hz, H-3'), 4.00 (1Hm, $J_{4'-5'a}$ = 3.12 Hz, $J_{4'-5'b}$ = 3.17 Hz, H-4'), 3.78 (2Hm, $J_{5'a-5'b}$ = 13.92 Hz, H-5'ab), 2.65 (1Hm, $J_{2'a-2'b}$ = 13.45 Hz, H-2'a), 2.31 (1Hm, H-2'b).

^{1}H (d^6-DMSO): δ: 7.32 (1Hs, H-2), 6.80 (1H broad s, NH), 6.72 (1H broad s, NH), 5.92 (1Hdd, $J_{1'-2'a}$ = 8.00 Hz, $J_{1'-2'b}$ = 6.00 Hz, H-1'), 5.32 (1Hd, 3'-OH), 5.16 (1Ht, 5'-OH), 4.31 (1Hm, H-3'), 3.80 (1Hdd, H-4'), 3.54 (2H broad t, H-5'ab), 2.40 (1Hm, H-2'a), 2.13 (1Hm, H-2'b).

^{13}C NMR (CD_3OD): δ: 40.35 (C-2'), 62.76 (C-5'), 72.41 (C-3'), 86.30 (C-1'), 89.00 (C-4'), 113.56 (C-4), 130.57 (C-2), 145.30 (C-5), 169.35 (amide CO).

Nuclear Overhauser enhancement experiments (Figures 2a and 2b) - Signal enhancements (%) caused by saturation of the signal at 6.03 ppm (H-1'): H-2 (3.4); H-2'b (5.4); H-4' (1.0). Signal enhancements (%)

caused by saturation of the signal at 7.2 ppm (H-2); H-1' (3.0); H-2'a (1.5).

RESULTS AND DISCUSSION

Using a mixture of deoxyribosyl transferases from *Lactobacillus leichmanii*[11], the transfer of 2-deoxyribose from thymidine to 5-aminoimidazole-4-carboxamide or from 2'-deoxycytidine to benzimidazole occurred in good yield. As benzimidazole has a symmetrical structure, there was no problem in assigning the position of attachment of the 2-deoxyribose residue. Using nuclear Overhauser enhancement techniques (Figure 1), the conformation of the glycosidic link in (3) was shown to be β. Saturation of the signal due to H-1' caused enhancement of the signal due to H-8 as well as the signals due to H-2'b and H'4' on the underside of the deoxyribose ring. No enhancements were observed for signal on the topside of the deoxyribose ring. When the signal due to H-3' was saturated, the signal due to H-8 was enhanced indicating that the base was in the "anti" conformation.

The assignment of the structure of compound (6) was more complex as there are four nitrogen atoms in (6) which could be the position of attachment of the 2-deoxyribose residue. Only one product could be detected by HPLC. Confirmation of the position of attachment of the deoxyribose residue was made by comparison of the ^{1}H NMR spectrum taken in d^6-DMSO of (6) with the published spectra of the isomeric 1-β-D-ribofuranosyl-4(5)-aminoimidazole-5(4)-carboxamides[8]. In the ^{1}H NMR spectrum of 1-β-D-ribofuranosyl-5-aminoimidazole-4-carboxamide the signal due to H-2 appeared at ca 7.3 ppm, whereas in 1-β-D-ribofuranosyl-4-aminoimidazole-5-carboxamide the corresponding signals were at 7.8 ppm. In compound (6), the signal due to H-2 is at 7.32 ppm. In the α- and β-anomers of 1-D-2'-deoxyribofuranosyl-4-aminoimidazole-5-carboxamide[8], the signals due to H-2 were at ca 7.8 ppm in each case (in agreement with the value found for the corresponding ribonucleoside).

The anomeric configuration of (6) was established by nuclear Overhauser enhancement experiments (Figure 2). As was the case with (3), saturation of the signal due to H-1' caused enhancement of signal due to H-2 in the imidazole ring as well as those due to H-2'b and H-4' on the underside of the deoxyribose ring. No enhancement of signals of protons on the topside of the ring, eg, H-2'a, H-3', or H-5' could be observed. Saturation of the signal due to H-2 caused enhancement of the signal due to H-1' as well as that due to H-2'a but not H-3' on the topside of the ring. This suggests that the nucleoside is ß-linked and that the imidazole ring lies at an angle to the deoxyribose ring as shown (Figure 2b).

The ß-configuration of compound (6) was further confirmed by a comparison of the shapes of the signal due to H-1' in its ^{1}H NMR spectrum (in DMSO) with the shapes due to H-1' in the published ^{1}NMR spectrum (in DMSO) of the α- and ß-anomers of 1-D-ribofuranosyl-4-aminoimidazole-5-carboxamide[8]. The ß-anomer of this ribonucleoside and our compound (6) showed a "pseudotriplet" for H-1' while the α-anomer of the ribonucleoside showed a quartet[17]. It is relevant that in the ^{1}H NMR spectra of the related 2'-deoxy-D-ribofuranosylthiazole-4-carboxamide, the ß-anomer showed a "pseudotriplet", while the α-anomer showed a quartet for H-1'[18].

The formation of only one compound in the deoxyribosyl transfer to 5-aminoimidazole-4-carboxamide suggests that hydrogen bonding in the active site of the enzyme may favour the imidazole residue being held in one position and that N-3 together with the 5-amino- and the 4-carboxamido-groups may be involved in this hydrogen bonding. We have observed[11] that only one product (9-ß-D-2'-deoxyribofuranosyl- 1-deazapurine) is formed in a similar glycosyl transfer to 1-deazapurine. No product arising from glycosylation at the 7-position can be detected suggesting that N-1 and N-7 in the deazapurine may be involved in hydrogen bonding which determines the position of the deazapurine residue in the active site. As the benzo-ring in benzimidazole lacks groups capable of forming hydrogen bonds with the active site of the transferase apart from N-3, other factors such as charge-transfer must be involving the benzo-ring may be important in binding benzimidazole to the active site.

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REFERENCES

1 "The Biochemistry of the Nucleic Acids", 9th ed, R L P Adams, R H Burdon, A M Campbell, D P Leader, and R M S Smellie, eds, Chapman and Hall, London, 1981, p180

2 B Estramareix and S David, Biochem Biophys Res Comm, 1986, 134, 1136; P C Newall and R G Tucker, Biochem J, 1968, 106, 279; K Yamada and H Kumaoka, J Nutr Sci Vitaminol, 1983, 29, 239

3 "Ribavirin: A Broad Spectrum Antiviral Agent", R A Smith and W Kirkpatrick, Academic Press, New York, 1980

4 O Makabe, H Suzuki and S Umezawa, Bull Chem Soc Japan, 1977 50, 2689

5 M Ikehara and T Inaoka, Nucleosides and Nucleotides, 1985, 4, 515

6 G Mackenzie, G Shaw, and S E Thomas, J Chem Soc, Chem Comm,1976, 453

7 M P Groziak, B Bhat, and N J Leonard, Proc Nat Acad Sci, USA, 1988, 85, 7174

8 L B Townsend, "Synthetic Procedures in Nucleic Acid Chemistry", Vol 2, W W Zorbach, and R S Tipson, eds, J Wiley, New York, 1973, p268

9 D Ewing, R W Humble, A Holy, G Mackenzie, G Shaw and I Votruba, Nucleic Acids Res., 1989, Symp Series 21, 97

10 H Vorbrüggen, K Krolikiewicz, and B Bennua, Chem Ber, 1981, 114, 1256

11 D Betbeder, D W Hutchinson and A O'L Richards, Nucleic Acids Res, 1988, 17, 4217

12 M C Huang, K Hatfield, A A Roetker, J A Montgomery, and R L Blakeley, Biochem Pharmacol, 1981, 30, 2663

13 M C Huang, J A Montgomery, M C Thorpe, E L Stewart, J A Secrist III, and R L Blakeley, Arch Biochem Biophys, 1983, 222, 133

14 H Shirae, K Yokozeki, and K Kubota, Agric Biol Chem, 1988, 52, 1233

15 J Holguin, R Cardinaud, and C A Salemink, Eur J Biochem, 1975, 54, 515

16 J A Montgomery and H J Thomas, J Med Chem, 1972, 15, 1334

17 M J Robins and R K Robins, J Amer Chem Soc, 1965, 87, 4934

18 P C Srivastava, R K Robins, F Takusagawa, and H M Berman, J Heterocycl Chem, 1981, 18, 1659

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