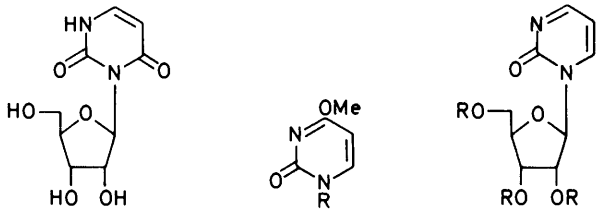
A New Synthesis of 3-(β-D-Ribofuranosyl)uracil (Isouridine) *via* the Intermediacy of an O^6,5'-Cyclotetrahydropyrimidinone Nucleoside

Kambhampati V. B. Rao, Victor E. Marquez,* James A. Kelley, and Mary T. Corcoran
Drug Design and Chemistry Section, Laboratory of Medicinal Chemistry and Biology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland 20205

A specific and efficient synthesis for 3-(β-D-ribofuranosyl)uracil, isouridine (1), has been devised. Starting with the easily accessible 1-(β-D-ribofuranosyl)-1,2-dihydropyrimidin-2-one (3b) the C-6 carbonyl function of isouridine was built *via* formation of the corresponding O^6, 5'-cyclonucleoside (5) followed by a two-step oxidation with *o*-chloranil and base-catalyzed hydrolysis of the intermediate 6-chloro-O^6,5'-cyclonucleoside (12a). The resulting isopropylideneisouridine (11) was easily deblocked to give the desired target (1). A 25% overall yield of (1) from pyrimidin-2-one and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose was obtained.

The unnatural isomer of uridine, 3-(β-D-ribofuranosyl)-uracil, isouridine (1), was characterized and synthesized over 20 years ago.[1,2] Surprisingly, however, there has been very little reported in relation to its biological activity. Moreover, a survey of the National Cancer Institute's data bank of compounds evaluated for antitumor activity revealed that isouridine had never been submitted for testing at this Institute. The most complete biological investigation of isouridine was conducted by Holý and his collaborators who studied the behaviour of the nucleotide derivative of (1), as well as its 2',3'-cyclic phosphate, with respect to some nucleolytic enzymes.[3-5] In addition, the same group found that in a reaction catalyzed by ribonucleases, (1) behaved as a good acceptor with adenosine 2',3'-cyclic phosphate and uridine 2',3'-cyclic phosphate to form the corresponding dinucleotide monophosphates.[6] It was also from Holý's laboratory that a new and specific synthesis for isouridine was devised.[7] This attractive synthesis avoided the formation of by-products typical of some previous syntheses and improved the overall yield with respect to other methodologies.[8,9] Holý's specificity for N-3 ribosylation was accomplished by the use of 1-(2-cyanoethyl)-4-methoxypyrimidin-2-one (2b) which was easily prepared from 4-methoxypyrimidin-2-one (2a). After the successful nucleosidation of (2b) with a protected halogeno-sugar, all protective groups were removed simultaneously to give isouridine (1) in 24% overall yield from (2a).[7] The synthesis failed, however, when the starting pyrimidinone carried a 5-methyl substituent.

We here report a new and efficient synthesis of isouridine which may also have the potential of being adaptable to 5-substituted N-3 ribofuranosyl derivatives. In addition, this new synthesis has provided two new intermediates (12a) and (12b), which are undergoing further biological investigation because of their unusual chemistry.



(1) (2) (3)

a ; R = H a ; R = Bz

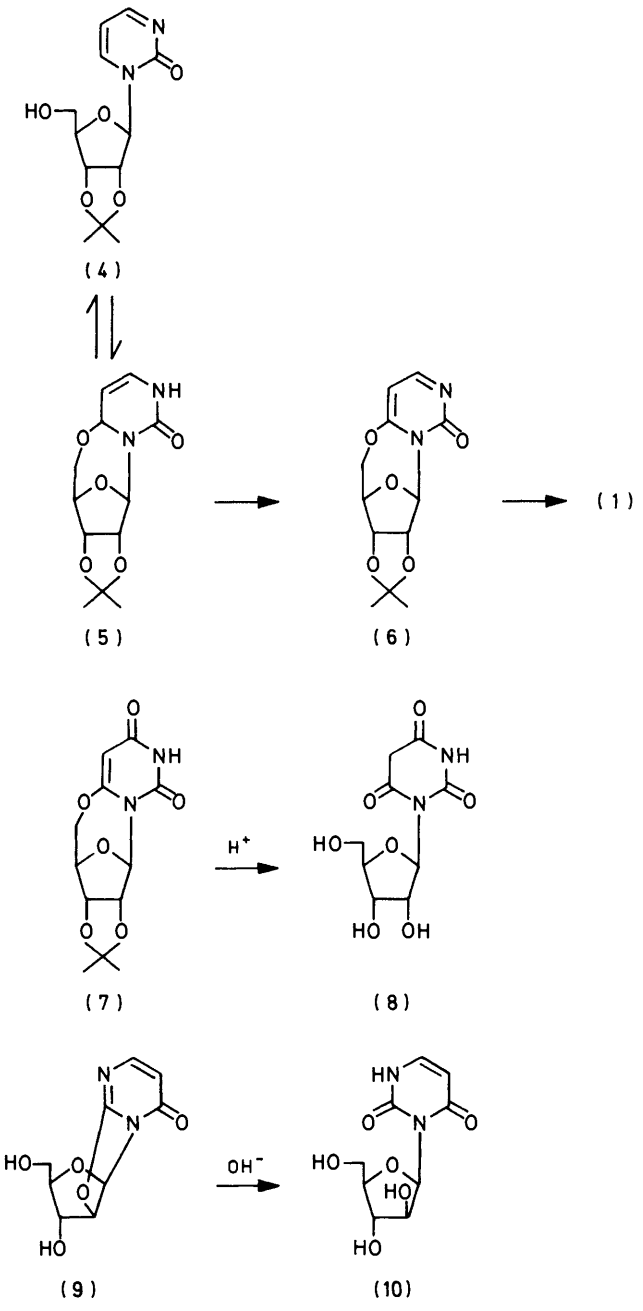
b ; R = CH_2CH_2CN b ; R = H

Results and Discussion

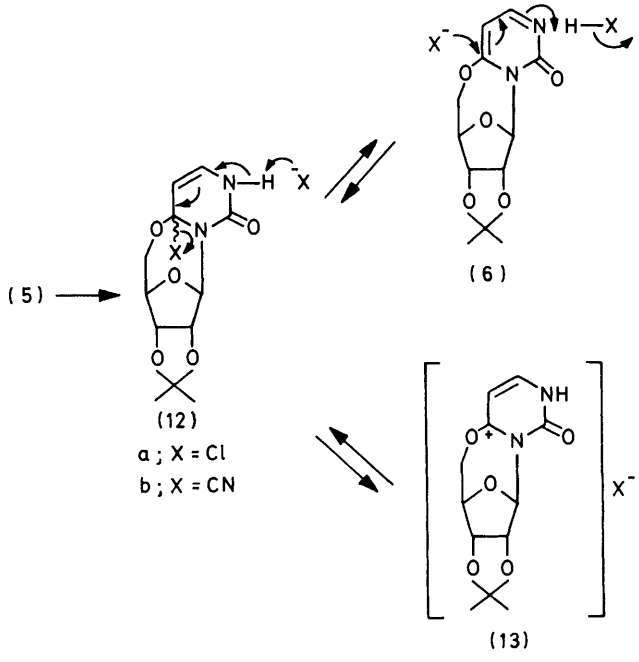
The key starting material of the synthesis was our previously reported cyclic nucleoside (5) which was readily obtained in two steps from the corresponding 2-pyrimidinone ribofuranosides (3a) and (3b) (see Experimental section).[10] Compound (5) existed in equilibrium with its open form (4) in aqueous solution as revealed by n.m.r. spectroscopy in D_2O. However, after lyophilization the material cyclized quantitatively back to (5) and remained as such in aprotic organic solvents. This O^6,5'-cyclo-isomer of the pyrimidin-2-one nucleoside was desired for further synthetic manipulation. Previously reported examples of chemical transformations with anhydronucleosides (7) and (9) indicated that oxidation of compound (5) to the stage of (6) might provide a simple one-step synthesis to our desired target (1) by a similar ring-opening process (Scheme 1).[11-13]

To that effect compound (5) was treated with dichlorodicyanobenzoquinone (DDQ). This oxidizing reagent has been used successfully for the dehydrogenation of dihydropyrimidin-2-ones to their corresponding pyrimidin-2-ones.[14] Compound (5) proved to be quite sensitive to DDQ under refluxing conditions in benzene and underwent extensive decomposition. However, when the reaction was performed at room temperature for 24 h in either chloroform or benzene, a low yield (10%) of a new product different from the expected (6) was isolated. This product proved to be (11), the isopropylidene derivative of (1), according to mass spectral and n.m.r. analyses. As evidenced from t.l.c. analyses performed at different intervals of time during the reaction, compound (11) was the only observable product in addition to starting material. The low yield of the product appeared to have been due to complexation between DDQ, or its reduced product, with a precursor of compound (11). The outcome of the reaction was the same even when moisture was carefully excluded.

o-Chloranil has also been reported to give good results in the oxidation of dihydropyrimidin-2-ones.[15] When this reagent was tried, the reaction proceeded smoothly, giving a good yield of a material different from either (6) or (11). As before, no traces of any other product were observed on t.l.c. during the entire reaction. The new isolated product was characterized by mass spectral and n.m.r. analyses as (12a). The mass spectral data was especially conclusive since it revealed the unequivocal presence of chlorine in compound (12a). This unusual compound obviously incorporated a chlorine atom from *o*-chloranil by an unknown mechanism. When the purity of the reagent was checked for the presence of chloride ion it proved to be negative. Therefore, instead of



Scheme 1



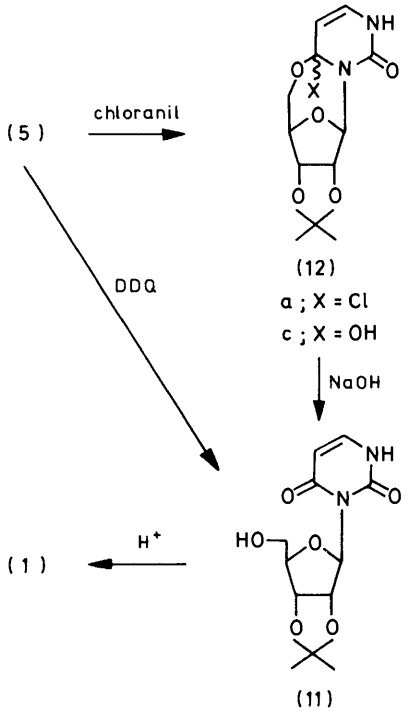
Scheme 2

the expected tetrachlorocatechol, a trichlorinated catechol must have been formed in this reaction to account for the liberation of Cl^- from *o*-chloranil. This aspect of the reaction has not yet been fully investigated. The identity of (12a), however, rests on solid ground as seen from the discussion to follow. The C–Cl bond in (12a) was expected to be fairly labile. Indeed, the chlorine atom was exchanged for CN after treatment of (12a) with KCN in anhydrous dimethyl sulphoxide to give (12b). It is surprising that such exchange would take place with preference over the aromatization pathway that leads to (6). Two explanations can be given to accommodate this result. Compound (6) could indeed be formed, but undergoes rapid addition of cyanide to give (12b). Alternatively, the reaction may proceed through the dissociation of (12a) to give the carbocation (13), followed by the exchange of chloride by cyanide. Both mechanisms are depicted in Scheme 2. In an effort to generate (6), according to the first possibility, (12a) was treated with a non-nucleophilic base such as sodium hydride in benzene. The reaction was followed by n.m.r. and formation of the anion was evidenced by the changes in the spectrum. However, even after prolonged heating, (12a) was recovered unchanged following cooling and neutralization of the reaction mixture with cation exchange resin. This result tends to support the intermediacy of (13) but it does not constitute a mechanistic proof. It was reasoned, therefore, that an exchange of chlorine by OH in (12a) would give a product (12c) that should spontaneously rearrange to (11).

As seen in Scheme 3 the expected rearrangement of (12c) was realized and (12a) was smoothly converted into (11) in 78% yield after treatment with 0.1M-NaOH at 65 °C for 20 h. Finally, compound (11) was cleanly deprotected to the final target by treatment with a strong cation exchange resin in water. The overall transformation starting from (5) is depicted in Scheme 3.

Isouridine obtained by this procedure was identical in every respect with that reported earlier in the literature.[2,8,9] The overall conversion starting with the condensation of pyrimidin-2-one with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose afforded yields in the range 20—25%. The method is simple and can be easily adapted for a large-scale operation. More importantly, as mentioned earlier, the synthesis could be adapted to produce 5-substituted isouridine derivatives. Owing to the symmetry of the 5-substituted pyrimidin-2-one to be used as the starting material, only one isomer would initially be obtained from the nucleosidation with the appropriate sugar furanoside in the condensation reaction. Later, the equilibrium resulting in the formation of an O^6,5′-cyclonucleoside analogous to (5) could be used to introduce the asymmetry characteristic of an isouridine moiety by the same procedure. The generality of this approach is currently being investigated in our laboratory.

When isouridine was tested for its inhibitory activity against P388 cells in culture, it showed no inhibition of cell



Scheme 3

growth at concentrations as high as 5×10^{-4}M. In addition, at 1×10^{-4}M, 1×10^{-3}M, and 1×10^{-2}M, it failed to inhibit uridine kinase extracted from P388 cells.

Experimental

General Methods.—M.p.s were determined on a Thomas-Hoover apparatus and are uncorrected. Specific rotations were measured in a 1-dm cell with a Perkin-Elmer Model 141 polarimeter. ^{1}H N.m.r. spectra were determined on Varian T-60 or HR-220 instruments. Chemical shifts are given as δ values with reference to $SiMe_4$ or deuteriated sodium 3-(trimethylsilyl)propionate (TSP). I.r. spectra were obtained as Nujol mulls in a Perkin-Elmer 727B spectrophotometer. Elemental analyses were carried out by Galbraith Laboratories, Inc., Knoxville, TN. Low-resolution electron-impact mass spectra (70 or 75 eV) were obtained on a DuPont 21-492B gas chromatograph-mass spectrometer (g.c./m.s.) system interfaced to a VG 2040 data system or Hitachi Perkin-Elmer RMU-6E spectrometer. Samples were introduced either by direct probe or *via* a Varian 2 740 gas chromatograph (trimethylsilyl derivatives) coupled to the mass spectrometer by a single-stage glass jet separator. Columns for chromatography were packed with silica gel (Bio-Sil A, 200—400 mesh, Bio-Rad Laboratories) or neutral alumina (Alumina Woelm, activity grade III) and eluted with the solvents indicated in the individual experiments. Preparative h.p.l.c. was performed on a Waters instrument prep LC/system 500A.

1-(2,3,5-*Tri*-O-*benzoyl*-β-D-*ribofuranosyl*)-1,2-*dihydropyrimidin*-2-*one* (3a).—This material was obtained by the method of Vorbrüggen *et al.* in yields of *ca.* 70%, m.p. 152—154 °C (EtOH) (lit.,[16] m.p. 155—158 °C).

1-β-D-*Ribofuranosyl*-1,2-*dihydropyrimidin*-2-*one* (3b).—This compound was obtained as reported previously after deblocking of the precursor (3a) with saturated methanolic ammonia.[10] The total yield of pure material was improved to 90%.

1-(2′,3′-O-*Isopropylidene*-β-D-*ribofuranosyl*)-O^6,5′-*cyclo*-1,2,3,6-*tetrahydropyrimidin*-2-*one* (5): *a New Improved Method.*—A suspension of (3b) (4.28 g, 18.75 mmol) in dry acetone (1 l) was stirred for 1 h at 25 °C with toluene-*p*-sulphonic acid monohydrate (53 g; previously dried *in vacuo*). The reaction mixture was poured into aqueous 0.5M-$NaHCO_3$ (1.2 l) and the resulting mixture lyophilized to dryness. The resulting solid was extracted with dry benzene and the benzene extracts concentrated under reduced pressure. The residue obtained was purified by preparative h.p.l.c. (silica gel) using ethyl acetate–hexane (4 : 1). The combined fractions containing the product were evaporated to yield (5) as a white foamy solid (3.27 g, 65%), m.p. 103—105 °C (lit.,[10] m.p. 104—106 °C).

6-*Chloro*-1-(2′,3′-O-*isopropylidene*-β-D-*ribofuranosyl*)-O^6,5′-*cyclo*-1,2,3,6-*tetrahydropyrimidin*-2-*one* (12a).—A solution of (5) (0.256 g, 0.95 mmol) in dry benzene (70 ml) was refluxed for 36 h under nitrogen in the presence of tetrachloro-*o*-benzoquinone (0.512 g, 2.08 mmol). The reaction mixture was concentrated under reduced pressure and the residue dissolved in ethyl acetate; the resulting solution was passed through a neutral alumina column (2 × 12 in) using ethyl acetate followed by methylene chloride–methanol (20 : 1) as eluant. The methylene chloride–methanol fractions were combined and concentrated under reduced pressure. The residue obtained was purified by flash chromatography using silica gel and ethyl acetate–hexane (4 : 1). The fractions containing the product were combined and evaporated to give (12a) as a white foamy solid (0.240 g, 83%), m.p. 94—96 °C; δ($CDCl_3$) 1.39 (s, 3 H, CH_3), 1.60 (s, 3 H, CH_3), 3.50—3.80 (m, 2 H, 5′-H, 5′a-H), 4.15—4.50 (m, 1 H, 4′-H), 4.80—5.20 (m, 2 H, 2′, 3′-H), 5.74 (d, 1 H, $J_{4,5}$ 8 Hz, 5-H), 6.56 (s, 1 H, 1′-H), 7.10 (dd, 1 H, $J_{4,5}$ 8 Hz, $J'_{3,4}$ 4 Hz, converted into a doublet, $J_{4,5}$ 8 Hz, after D_2O exchange, 4-H), and 9.92br (d, 1 H, $J_{3,4}$ 4 Hz, NH, D_2O exchanged); mass spectrum as a trimethylsilyl derivative, *m/z* (rel intensity) 374 ($M^{+\cdot}$, 0.3), 361 ($^{37}ClM - CH_3$, 4.8), 359 ($^{35}ClM - CH_3$, 12), 339 (M − Cl, 2.5), 299 (7.1), 281 (13), 251 (13), 209 (11), 185 (100), 169 (39), 132 (23), 96 (20), 73 (39), 68 (12), and 43 (34) (Found: C, 47.75; H, 5.01; N, 9.03. Calc. for $C_{12}H_{15}ClN_2O_5$: C, 47.61; H, 4.99; N, 9.26).

3-(2′,3′-*o*-*Isopropylidene*-β-D-*ribofuranosyl*)*uracil* (11).—*Method A.* A solution of (12a) (3.62 g, 11.29 mmol) in aqueous 0.1M-NaOH (108 ml) was heated at 65 °C with continuous stirring for 22 h. The reaction mixture was then cooled with ice and neutralized with pre-washed strong cation-exchange resin (50W-X8, 100—200 mesh, Bio-Rad). The resulting mixture was filtered through Celite and the filtrate was lyophilized to give crude product (2.8 g). This material was purified by flash chromatography using silica gel and ethyl acetate. The fractions containing the product were combined and evaporated under reduced pressure to give (11) as a white foamy solid (2.5 g, 78%), m.p. 90—92 °C; δ($CDCl_3$) 1.30 (s, 3 H, CH_3), 1.51 (s, 3 H, CH_3), 3.73 (m, 2 H, 5′-H, 5′a-H), 4.10 (m, 1 H, 4′-H), 4.88 (m, 1 H, 3′-H), 5.03 (m, 1 H, 2′-H), 5.19br (s, 2 H, NH and OH, D_2O exchanged), 5.54 (d, 1 H, $J_{4,5}$ 8 Hz, 5-H), 6.35 (d, 1 H, $J_{1',2'}$ 2.5 Hz, 1′-H), 7.07 (d, 1 H, $J_{4,5}$ 8 Hz, 4-H); mass spectrum, *m/z* (rel intensity) 269 (M − 15, 11), 251 ($M - CH_2OH$, 6.6), 226 ($M - CH_3COCH_3$, 1.3), 195 (4.5), 179 (7.3), 157 (5.5), 137 (21), 113 (44), 112 (35), 69 (51), 59 (55), and 43 (100)

(Found: C, 50.95; H, 6.1; N, 9.5. Calc. for $C_{12}H_{16}N_2O_6$: C, 50.70; H, 5.67; N, 9.86).

Method B. A suspension of (5) (2.01 g, 7.5 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (1.88 g, 8.28 mmol) in chloroform (150 ml) was stirred at 25 °C for 24 h. The reaction mixture was passed through a short neutral alumina column and eluted with methylene chloride–methanol (8 : 1). Fractions containing (11) were combined and evaporated to give pure product (0.096 g). The remaining fractions were combined and rechromatographed with neutral alumina using in succession methylene chloride, acetone, and methylene chloride–methanol as eluants to give pure (11) (0.124 g) plus starting material (5) (0.050 g); the total yield of (11) was 0.22 g (10%), m.p. 94—96 °C. The chemical and physical properties of this compound were identical with that obtained by method A.

3-(β-D-*Ribofuranosyl*)*uracil* (1).—A mixture of (11) (1.0 g, 3.52 mmol) and pre-washed cation exchange resin (50W-X8, 100—200 mesh, Bio-Rad) in water (60 ml) was stirred at 25 °C for 15 h. The mixture was filtered, cooled, and neutralized with dilute NH_4OH. The resulting solution was mixed with charcoal, filtered, and lyophilized to give (1) as a white fluffy solid (0.694 g, 80%). This solid was recrystallized from absolute alcohol to give (1) (0.58 g, 67%), m.p. 198—200 °C (lit.,[9] m.p. 199—201 °C); $[\alpha]_D^{23}$ −27.9° (*c* 0.082, H_2O); δ(D_2O) 3.90 (m, 3 H, 4′-H, 5′-H, 5′a-H), 4.42 (t, 1 H, 3′-H), 5.80 (d, 1 H, $J_{4,5}$ 8 Hz, 5-H), 6.25 (d, 1 H, $J_{1',2'}$ 3 Hz, 1′-H), and 7.48 (d, 1 H, $J_{4,5}$ 8 Hz, 4-H); mass spectrum as a tetrakis-(trimethylsilyl) derivative, *m*/*z* (rel. intensity) 517 (*M* − 15, 3), 427 (0.5), 387 (2.2), 371 (1.3), 348 (8.9), 315 (20), 245 (39), 217 (29), 185 (10), 169 (17), 147 (35), 103 (9), and 73 (100).

6-*Cyano*-1-(2′,3′-O-*isopropylidene*-β-D-*ribofuranosyl*)-O⁶,5′-*cyclo*-1,2,3,6-*tetrahydropyrimidin*-2-*one* (12b).—A solution of (12a) (0.1 g, 0.33 mmol) in anhydrous dimethyl sulphoxide (10 ml) was treated with potassium cyanide (0.1 g, 1.56 mmol) at 65 °C for 24 h. The reaction mixture was then poured in ice–water (*ca.* 100 ml) and extracted with chloroform (3 × 20 ml) and ethyl acetate (3 × 20 ml). The combined organic extracts were dried (Na_2SO_4), filtered, and reduced to dryness. The yellowish residue was purified by preparative t.l.c. using three 2 000 μ silica gel plates (20 × 20 cm) developed with ethyl acetate–hexane (4 : 1). Pure (12b) (0.041 g, 42%) was isolated as a fluffy solid, m.p. 110—112 °C, $\nu_{max.}$ (Nujol) 2 270 cm^{-1} (CN); δ($CDCl_3$) 1.35 (s, 1 H, CH_3), 1.56 (s, 1 H, CH_3), 2.83 * (d, 2 H, $J_{4',5'}$ 7 Hz, 5′-H, 5′a-H), 4.00—4.40 (m, 1 H, 4′-H), 4.90 (dd, 1 H, $J_{2',3'}$ 6 Hz, $J'_{3',4'}$ 4 Hz, 3′-H), 5.15 (d, 1 H, $J_{2',3'}$ 6 Hz, 2′-H), 5.72 (d, 1 H, $J_{4,5}$ 8 Hz, 5-H), 6.55 (s, 1 H, 1′-H), 7.20 (d, 1 H, $J_{4,5}$ 8 Hz, 4-H), 9.30—10.10br (s, 1 H, NH, D_2O exchanged); *m*/*z* 278 (*M* − 15) (Found: C, 53.15; H, 5.3; N, 14.1. Calc. for $C_{13}H_{15}N_3O_5$: C, 53.22; H, 5.15; N, 14.32).

* These hydrogens lie in the shielding cone of the CN group. From molecular models it can be seen that this only happens when the CN at C-6 is ' down.' Since we are dealing with a single isomer the stereochemistry at this position might be correctly assigned from the n.m.r. data.

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