Synthesis of 2′- and 3′-*O*-Acyluridines

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A method for the synthesis of 2′- and 3′-*O*-acyluridines is described. It involves the preparation of 2′,5′- and 3′,5′-di-*O*-benzyluridines as key intermediates, and is exemplified by the preparation of 2′- and 3′-*O*-pivaloyluridine. An attempt to prepare 2′- and 3′-*O*-L-valyluridine led to a mixture of isomers; interconversion occurred during the final stage. The possible utility in an oligoribonucleotide synthesis of 5′-*O*-acetyl-2′-*O*-trityluridine, an intermediate in the synthesis of 3′,5′-di-*O*-benzyluridine, is discussed.

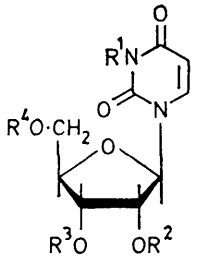
A SYNTHESIS of the mixed 2′- and 3′-*O*-(1-aminocyclopentylcarbonyl)-adenosines and -uridines has been reported.[1] A general method was then required whereby 2′- and 3′-*O*-acyl derivatives of adenosine or uridine could be separately prepared. Interest in such derivatives was prompted by the tentative suggestion [1] that hydroxy-group-assisted acyl migration might be sterically hindered in the 1-aminocyclopentylcarbonyl derivatives, and by implication, for similarly bulky acyl substituents. Of particular interest, therefore, were derivatives containing a similarly large acyl substituent, such as pivaloyl, or a bulky naturally occurring aminoacyl substituent, such as valyl.

Synthetic routes to 2′- and 3′-*O*-acylribonucleosides have involved either acid-catalysed hydrolysis of an appropriate 2′,3′-substituted cyclic orthoester [2] or partial acylation of a ribonucleoside suitably protected at the 5′-position.[3,4] Both methods have yielded initially mixtures of the 2′- and the 3′-monoacylribonucleosides, from which isolation of pure samples of both isomers requires that they be chromatographically distinguishable and isolable under conditions not conducive to acyl group migration. Uniquely, 2′- and 3′-*O*-benzoyl-6,6-di-*N*-methyladenosines satisfy these conditions, and have been separated from an acid hydrolysate of the appropriate 2′,3′-substituted orthoester.[5]

Although useful investigations into acyl group migration have been carried out on non-equilibrium mixtures of 2′- and 3′-*O*-acylribonucleosides,[4] the use of pure isomers for this purpose is desirable in cases where neither isomer has previously been characterized individually. A general synthesis of a pair of such isomers would require a pair of intermediates with selectively removable substituents on the primary and on the appropriate secondary position of the ribose system. Acid-labile or hydrogenolytically removable protecting groups are the obvious choice for the synthesis of the base-labile acyl derivatives. Uridine was selected for an initial evaluation of protecting groups. This choice obviated the need for additional protection of nucleophilic sites on the base residue.

One pair of potentially suitable protected uridine derivatives was immediately accessible. 2′,5′- and 3′,5′-di-*O*-trityluridine have been prepared in one stage from uridine.[6] However, 2′,5′-di-*O*-trityluridine was resistant to acylation by *N*-benzyloxycarbonyl-L-valine anhydride. Thus trityl groups appear to be unsatisfactory for the present purpose, presumably on account of their bulkiness. An alternative, the benzyl function, is unlikely to impede electrophilic attack at a neighbouring hydroxy-function.

The value of selective protection by benzyl in acylnucleoside synthesis was demonstrated by Reese and Trentham.[7] They used 3′- and 2′-*O*-benzyluridine to make isomeric 2′,5′- and 3′,5′-di-*O*-acyluridines. It therefore seemed that 3′,5′- and 2′,5′-di-*O*-benzyluridine, (I) and (II), would serve as suitable intermediates for the preparation of 2′- and 3′-*O*-acyluridines. Moreover, the syntheses described by Reese and Trentham (benzylation of the aforementioned 2′,5′- and 3′,5′-di-*O*-tritylurídines followed by acid-catalysed removal of the trityl groups) suggested a route to the dibenzyluridines from 2′- and 3′-*O*-tritylurídines, (III) and (IV).



	R^1	R^2	R^3	R^4
(I)	H	H	$PhCH_2$	$PhCH_2$
(II)	H	$PhCH_2$	H	$PhCH_2$
(III)	H	Ph_3C	H	H
(IV)	H	H	Ph_3C	H
(V)	H	Ph_3C	H	Ac
(VI)	H	H	Ph_3C	Ac
(VII)	H	Ph_3C	Ph_3C	Ac
(VIII)	H	Ph_3C	$PhCH_2$	$PhCH_2$
(IX)	H	$PhCH_2$	Ph_3C	$PhCH_2$
(X)	H	Z-Val	$PhCH_2$	$PhCH_2$
(XI)	H	$PhCH_2$	Z-Val	$PhCH_2$
(XII)	H	Val	H	H
(XIII)	H	H	Val	H
(XIV)	H	Piv	$PhCH_2$	$PhCH_2$
(XV)	H	$PhCH_2$	Piv	$PhCH_2$
(XVI)	H	Piv	H	H
(XVII)	H	H	Piv	H

Z-Val = *N*-benzyloxycarbonyl-L-valyl; Piv = pivaloyl

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[2] H. P. M. Fromageot, B. E. Griffin, C. B. Reese, and J. E. Sulston, *Tetrahedron*, 1967, **23**, 2315.
[3] J. Zemlicka, S. Chladek, Z. Haladova, and I. Rychlik, *Coll. Czech. Chem. Comm.*, 1969, **34**, 3755.
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[6] N. C. Yung and J. J. Fox, *J. Amer. Chem. Soc.*, 1961, **83**, 3060.
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An initial approach to the trityl derivatives (III) and (IV) was the partial hydrogenolysis of the corresponding ditrityluridines. The conversion of 1,3(or 2),5-tri-*O*-trityl-D-ribose into 1,3(or 2)-di-*O*-trityl-D-ribose by this procedure [8] provided a precedent. Although 2′-*O*-trityluridine (III) was thus prepared, albeit in poor yield, from 2′,5′-di-*O*-trityluridine, the sole monotrityluridine detected on thin-layer chromatograms of the products of partial hydrogenolysis of the 3′,5′-isomer was later shown to be 5′-*O*-trityluridine.

In the ultimately successful route, 5′-*O*-acetyluridine was converted into a mixture of 5′-*O*-acetyl-2′-*O*-trityluridine (V), its 3′-*O*-trityl isomer (VI), and 5′-*O*-acetyl-2′,3′-di-*O*-trityluridine (VII). The three products were readily separable by column chromatography. Base-catalysed removal of the acetyl groups from the mono-*O*-trityl derivatives (V) and (VI) gave 2′-*O*-trityluridine (III) and its 3′-isomer (IV) which, unlike their 5′-acetates, were inseparable by thin-layer and column chromatography.

Reaction of the trityluridines (III) and (IV) separately with benzyl chloride in refluxing dioxan in the presence of potassium hydroxide led to the di-*O*-benzyl derivatives (VIII) and (IX), easily separable from a number of by-products formed, presumably, by benzylation of the base residue. Hydrolytic detritylation of compounds (VIII) and (IX) led respectively to 3′,5′-di-*O*-benzyluridine (I) and its 2′,5′-isomer (II) which, by subsequent reaction with *N*-benzyloxycarbonyl-L-valine anhydride, gave the corresponding *N*-benzyloxycarbonyl-L-valyl derivatives (X) and (XI). The monoacetates of the valyluridines produced by hydrogenolysis, in 80% acetic acid, of each of these products gave identical n.m.r. spectra (in D_2O solution, 'pD' 3·9). The presence of both isomers was indicated [9] by the shoulders on the peaks of the H-6 doublet in spectra of solutions in [$^2H_6$]dimethyl sulphoxide. That migration had occurred during hydrogenolysis was shown by conducting the reaction in deuterioacetic acid–deuterium oxide.

In the suggested mechanism for the acid-catalysed hydroxy-group-assisted migration of the acyl group,[10] nucleophilic attack on the carbonyl carbon atom by the oxygen atom of the adjacent hydroxy-group is the rate-determining step. Hence any factor which reduces the electron density on this carbon atom will enhance the rate of this process. Rammler and Khorana assumed, but did not demonstrate, that rapid migration of the DL-phenylalanyl group between the 2′- and the 3′-positions on adenosine was promoted by the inductive effect of the protonated aminoacyl groups,[11] a view supported by McLaughlin and Ingram in a study of the L-valyladenosines.[12]

When the dibenzyl derivatives (X) and (XI) were hydrogenated in an anhydrous, neutral solvent (dioxan) in an attempt to avoid migration of the valyl group, extensive reduction of the uracil system occurred. The mass spectrum of the basic hydrolysate of the hydrogenation product lacked the peaks at *m/e* 113 (uracil residue + 2) and 141 (uracil residue + 30) characteristic of uridine,[13] but contained signals at 115 and 143. It was identical to the spectrum of an authentic sample of dihydrouridine. The loss of unsaturation precluded the use of the H-6 signal in the n.m.r. spectrum to determine whether or not migration of the aminoacyl group had occurred.

The route just described was then successfully applied to the synthesis of 2′- and 3′-*O*-pivaloyluridine [(XVI) and (XVII)]. Pivaloyl chloride reacted readily with each of the dibenzyluridines (I) and (II), showing that steric hindrance to the insertion of the bulky groups was not significant from the preparative point of view. Hydrogenolysis in dioxan of the products [(XIV) and (XV)] was faster than that of the corresponding valyl derivatives [(X) and (XI)] and gave the required pivaloyluridines without detectable reduction of the pyrimidine ring. No interconversion of the isomers was observed under these conditions. Base-catalysed interconversion of the products was demonstrated. Overlap often occurs between the H-5 and the H-1′ signals in the n.m.r. spectra of uridine derivatives.[9] Although the H-5 and H-1′ signals were well separated in the spectra of the two pivaloyluridines (XVI) and (XVII), overlap occurred between the signals for H-1′ and H-2′ of the 2′-isomer. The lower-field H-6 doublet has been used to estimate the relative proportions of 2′- and 3′-*O*-acyluridines in equilibrating mixtures.[14] The relative heights of the signals for the two isomers afforded a measure of the percentage of each in such mixtures. In the present study, the difference in chemical shift between the t-butyl singlets in the n.m.r. spectra of the two derivatives (XVI) and (XVII) provided an additional, and more useful parameter whereby equilibration could be followed. In a 0·03M-solution of morpholine in deuteriomethanol at 50°, the process was complete within 5 days. Under comparable (but not identical) conditions, equilibration of the corresponding 2′- and 3′-acetates occurred within 5 min.[15]

Complete equilibration of the pivaloyl derivatives was not accompanied by solvolysis. Hence, although no quantitative estimate was made of the relative rates of the two processes, the present studies afford no experimental evidence that steric hindrance to acyl group migration is significant. These pivaloyl derivatives are better models than the previously mentioned

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1-aminocyclopentanecarboxylates [1] for a study of the effect, if any, of steric factors on acyl group migration. Reese and his co-workers have pointed out that solvolysis of the labile 2'- and 3'-*O*-aminoacylribonucleosides during migratory studies using n.m.r. spectroscopy could lead to a false estimate of the percentage of the 3'-isomer in a mixture, since the H-1' signals of this isomer and those of the free ribonucleoside can be nearly coincident.[14]

Finally, one of the intermediates in the synthesis of the dibenzyluridines, 5'-*O*-acetyl-2'-*O*-trityluridine (V) has potential utility in the synthesis of oligoribonucleotides by the approach used by Reese and his co-workers.[16] Their route requires a ribonucleoside protected at the 2'- and at the 5'-position with, respectively, an acid-labile and a base-labile substituent. The present synthesis of 5'-*O*-acetyl-2'-*O*-trityluridine (V) constitutes a shorter path to a derivative of this type than any hitherto reported. Protection of the 3'-position, usual before insertion of the acid-labile substituent, is here unnecessary. The bulky 2'-*O*-trityl group hinders the extensive formation of the dityrityl derivative (VII) and, as previously mentioned, the 2'-*O*-trityl derivative (V) is fortuitously separable from its 3'-analogue (VI).

Although steric hindrance to phosphorylation at the 3'-position might be anticipated were a 2'-trityl substituent present, Ukita and co-workers have successfully phosphorylated 2',5'-di-*O*-trityluridine with a suitably protected uridine 5'-phosphate derivative.[17] The suitability of the 2'-trityl protecting group from the viewpoint of its lability to acid cannot be so directly assessed. The reagent used by Ukita *et al.*[17] for the removal of the trityl groups from fully protected uridylyl(3' ⟶ 5')-uridine, 80% acetic acid, promoted extensive migration between the 3'- and the 2'-positions of the internucleotide linkage. The use of hydrochloric acid for the removal of acid-labile protecting groups minimises this isomerisation.[16] It is likely that this reagent could remove a trityl group from the 2'-position of a protected oligoribonucleotide without causing significant migration of the internucleotide linkages.

EXPERIMENTAL

N.m.r. spectra were measured with a Perkin-Elmer R10 spectrometer, operating at 60 MHz, for *ca.* 10% w/v solutions in deuteriochloroform, unless otherwise indicated. Internal standards were acetonitrile in D_2O solutions and otherwise tetramethylsilane. Mass spectra were obtained, by direct insertion, on an A.E.I. MS-12 spectrometer, operating at 70 eV (source temperature 120—170°; trap current 100 μA).

All reactions were monitored by t.l.c.

Thin-layer chromatograms were run on plates (7·6 × 2·5 cm, 8 × 8 cm, and 20 × 5 cm) coated with silicic acid (Merck Kieselgel GF_{254}) or (where indicated) cellulose powder (Avicel No. 144 LS/254). Routine detection of spots was achieved with a Hanovia Chromatolite u.v. lamp. Valyl compounds were additionally detected with ninhydrin (aerosol in n-butanol). The method of Viscontini *et al.*[18] was used to detect *cis*-diols on chromatograms (*N.B.* Care must be taken in the application of this procedure, which employs benzidine, a highly carcinogenic compound; spraying should be done only in an efficient fume cupboard). Silicic acid for column chromatography was Merck Kieselgel (70—325 mesh). Dry solvents were distilled from calcium hydride. M.p.s were determined with a Kofler hot-stage apparatus, and are corrected.

Partial Hydrogenolysis of 2',5'- and 3',5'-Di-O-trityluridines.—2',5'-Di-*O*-trityluridine [6] (0·50 g, 0·69 mmol) was hydrogenated in ethyl acetate–ethanol (1 : 2; 45 ml) over 5% palladium–charcoal (0·5 g). After 50 h, four components were present: R_F (4% methanol–chloroform) 0·05, 0·18, 0·28, and 0·65 (starting material). The products R_F 0·18 and 0·05 gave a positive *cis*-diol test. The former was chromatographically identical with 5'-*O*-trityluridine, and the latter with uridine. The filtered solution was concentrated and a solution of the residue, in chloroform, was applied to a column of silicic acid (200 g), which was eluted with 2% v/v methanol–chloroform. Crystallization from ethanol of the component of R_F 0·28 gave 2'-O-*trityluridine* (III) (0·082 g, 25%), m.p. 230—231° (Found: C, 68·8; H, 5·5; N, 5·5. $C_{28}H_{26}N_2O_6$ requires C, 69·1; H, 5·4; N, 5·8%).

3',5'-Di-*O*-trityluridine,[6] similarly hydrogenated, gave only three significant components after 40 h; R_F (4% methanol–chloroform) 0·05 (uridine), 0·18 (*cis*-diol positive), and 0·60 (starting material). No product of R_F *ca.* 0·28 (3'-trityluridine; see before) was observed.

Tritylation of 5'-O-Acetyluridine.—A solution of 5'-*O*-acetyluridine [2] (1·00 g, 3·5 mmol) and chlorotriphenylmethane (1·70 g, 6·1 mmol) in dry pyridine (10 ml) was heated under reflux for 4 h. The cooled solution was poured into ice–water and the precipitate extracted with chloroform. The concentrated extract was re-concentrated with toluene (3 × 7 ml) and a solution of the concentrate in chloroform extracted with 5% w/v aqueous cadmium chloride (2 × 7 ml). The dried ($MgSO_4$) organic phase contained three components (t.l.c. in chloroform), which were consecutively eluted with chloroform from a column of silicic acid (120 g). 5'-O-*Acetyl*-2',3'-di-O-*trityluridine* (VII), R_F 0·32, gave crystals (0·100 g, 4%), m.p. 140—142° (from ethanol) (Found: C, 76·6; H, 5·7; N, 3·8. $C_{49}H_{42}N_2O_7$ requires C, 76·4; H, 5·5; N, 3·6%), τ 1·30 (1H, s, H-6), 2·85 (3OH, m, trityl H), 4·48 (1H, s, H-1'), 4·60 (1H, s, H-5), and 8·19 (3H, s, Ac). 5'-O-*Acetyl*-2'-O-*trityluridine* (V), R_F 0·22, gave crystals (0·66 g, 35%), m.p. 172—174° (from ethanol–di-isopropyl ether) (Found: C, 67·8; H, 5·5; N, 5·5. $C_{30}H_{28}N_2O_7$ requires C, 68·2; H, 5·3; N, 5·3%), τ 2·65 (16H, m, trityl H + H-6), 3·71 (1H, d, $J_{1',2'}$ 7·0 Hz, H-1'), 4·32 (1H, d, $J_{5,6}$ 8·5 Hz, H-5), and 8·00 (3H, s, Ac). 5'-O-*Acetyl*-3'-O-*trityluridine* (VI), R_F 0·15, gave crystals (0·71 g, 37%), m.p. 126—128° (Found: C, 68·0; H, 5·5; N, 5·5%), τ 2·53 (16H, m, trityl H + H-6), 4·12 (1H, d, $J_{1',2'}$ 5·0 Hz, H-1'), 4·30 (1H, d, $J_{5,6}$ 8·5 Hz, H-5, and 8·08 (3H, s, Ac).

2'-O-*Trityluridine* (III).—A solution of 5'-*O*-acetyl-2'-*O*-trityluridine (V) (1·51 g, 2·9 mmol) in saturated methanolic ammonia (15 ml) was kept at room temperature for 40 h, then concentrated. Crystallization from ethanol gave

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[18] M. Viscontini, D. Hoch, and P. Karrer, *Helv. Chim. Acta*, 1955, **38**, 642.

the 2′-trityl derivative (0·96 g, 70%) (Found: C, 68·7; H, 5·6; N, 5·8%), identical (t.l.c., m.p., and n.m.r.) with that prepared by partial hydrogenolysis of 2′,5′-di-*O*-trityluridine (see before).

3′-O-*Trityluridine* (IV).—5′-*O*-acetyl-3′-*O*-trityluridine (VI) (1·80 g, 3·4 mmol) was similarly treated. Crystallization from ethanol–petroleum (b.p. 60—80°) gave the *3′-trityl derivative* (1·14 g, 68%), m.p. 129—131° (Found: C, 68·7; H, 5·5; N, 6·0%), τ 2·62 (16H, m, trityl H + H-6), 4·27 (1H, d, $J_{1',2'}$ 5·0 Hz, H-1′), and 4·45 (1H, d, $J_{5,6}$ 7·0 Hz, H-5).

3′,5′-*Di*-O-*benzyl*-2′-O-*trityluridine* (VIII).—The reaction conditions, applied to 2′-*O*-trityluridine (III) (1·00 g, 2·1 mmol), were those used by Michelson and Todd [19] to convert 5′-*O*-trityluridine into its 2′,3′-di-*O*-benzyl derivative. The product was chromatographed on a column of silicic acid (150 g) with ether–petroleum (b.p. 60—80°) (2 : 1) as eluant. Elution of the *dibenzyl trityl derivative*, R_F (ether–petroleum, 2 : 1) 0·12 (0·33 g), which crystallized from the eluate as white needles, m.p. 189—190° (Found: C, 75·7; H, 6·1; N, 4·2. $C_{42}H_{38}N_2O_6$ requires C, 75·7; H, 5·7; N, 4·2%), was preceded by the elution of several by-products (total wt. 0·95 g).

3′,5′-*Di*-O-*benzyluridine* (I).—A solution of 3′,5′-di-*O*-benzyl-2′-*O*-trityluridine (VIII) (0·675 g, 1·0 mmol) in 80% acetic acid (4 ml) was heated under reflux for 1 h, then concentrated. Elution from a column of silicic acid (60 g) with ether gave triphenylmethanol followed by the *dibenzyl derivative*, obtained after concentration of the eluate as white needles (0·287 g, 67%), m.p. 68—69° (Found: C, 64·8; H, 5·9; N, 6·9. $C_{23}H_{24}N_2O_6$ requires C, 65·1; H, 5·7; N, 6·6%), τ 2·24 (1H, d, $J_{6,5}$ 9·0 Hz, H-6), 2·63 (10H, m, benzyl aromatic H), 4·14 (1H, d, $J_{1',2'}$, 4·0 Hz, H-1′), 4·62 (1H, d, H-5), and 5·34 and 5·49 (both 2H, s, benzyl CH_2), M^+ 424.

2′,5′-*Di*-O-*benzyluridine* (II).—The foregoing procedure was applied to 2′,5′-di-*O*-benzyl-3′-*O*-trityluridine (IX) (0·813 g, 1·2 mmol), obtained from 3′-*O*-trityluridine (IV) (0·93 g, 1·9 mmol) as a chromatographically homogenous glass by the method used to prepare the 2′-trityl analogue (VIII). The yield of *dibenzyluridine*, a white solid, m.p. 46—48°, was 0·41 g [79% based on (IX)] (Found: C, 64·7; H, 5·8; N, 6·3%), τ 2·18 (1H, d, $J_{6,5}$ 9·0 Hz, H-6), 2·67 (10H, m, benzyl aromatic H), 3·91 (1H, d, $J_{1',2'}$ 2·5 Hz, H-1′), 4·74 (1H, d, H-5), and 5·28 and 5·41 (both 2H, s, benzyl CH_2), M^+ 424.

3′,5′-*Di*-O-*benzyl*-2′-O-(N-*benzyloxycarbonyl*-L-*valyl*)*uridine* (X).—A solution of 3′,5′-di-*O*-benzyluridine (I) (0·200 g, 0·47 mmol) and *N*-benzyloxycarbonyl-L-valine anhydride [11] (0·456 g, 0·94 mmol) in dry pyridine (7 ml) was kept at room temperature. After 9 days, the solution was concentrated, then a solution of the residue in toluene was concentrated (×3). A solution of the residue in ether was applied to a column of silicic acid (40 g). Elution with ether gave the *acyl aminoacyl derivative*, a white solid (0·28 g, 90%), m.p. 55—56° (Found: C, 66·1; H, 6·2; N, 6·5. $C_{36}H_{39}N_3O_9$ requires C, 65·7; H, 6·0; N, 6·4), τ 2·28 (1H, d, $J_{6,5}$ 8·5 Hz, H-6), 2·70 (15H, m, benzyl aromatic H), 3·98 (1H, d, $J_{1',2'}$ 2·5 Hz, H-1′), 4·58—4·74 (2H, m, H-5 and H-2′), 4·91 and 5·59 (2H and 4H, respectively, s, benzyl CH_2), and 9·05 and 9·18 (both 3H, d, *J* 7·0 Hz, valyl CH_3).

2′,5′-*Di*-O-*benzyl*-3′-O-(N-*benzyloxycarbonyl*-L-*valyl*)*uridine* (XI).—2′,5′-Di-*O*-benzyluridine (II) was similarly converted into the 3′-*analogue*, a white solid (81%), m.p. 52—54° (Found: C, 65·9; H, 6·3; N, 6·6), τ 2·50—2·92 (16H, m, benzyl aromatic H + H-6), 3·96 (1H, d, $J_{1',2'}$ 6·0 Hz, H-1′), 4·70—4·82 (2H, m, H-5 and H-3′), 4·88 and 5·52 (2H and 4H, respectively, s, benzyl CH_2), and 9·01 and 9·13 (both 3H, d, *J* 7·0 Hz, valyl CH_3).

Hydrogenolysis of 2′,5′-*Di*-O-*benzyl*-3′-O-(N-*benzyloxycarbonyl*-L-*valyl*)*uridine*.—(*a*) The protected aminoacyl derivative (XI) (0·170 g, 0·26 mmol) was hydrogenolysed in 80% acetic acid (4 ml) over 5% palladium–charcoal (0·20 g) for 6 h. The filtered solution was lyophilized and gave the amorphous hygroscopic *monoacetates* of 2′- and 3′-O-L-*valyluridine* (0·090 g, 87%), R_F (ethyl acetate–ethanol, 1 : 2) 0·36, giving a negative *cis*-diol test and a positive ninhydrin reaction (Found: C, 47·1; H, 6·3; N, 10·1. Calc. for $C_{14}H_{21}N_3O_7,C_2H_4O_2$: C, 47·6; H, 6·3; N, 10·4%), τ (D_2O; ' pD ' 3·9) 2·12 (1H, d, $J_{6,5}$ 8·5 Hz, H-6), 3·98—4·17 (3H, m, H-1′, H-5, and H-2′), 8·07 (3H, s, Ac), and 8·95 (6H, d, *J* 6·0 Hz, valyl CH_3). A solution from which the internal standard (MeCN) had been omitted was used to obtain the integration of the acetate signal, which otherwise overlapped that of the standard.

(*b*) Reaction (*a*) was conducted in $CD_3{\cdot}CO_2D$–D_2O (4 : 1), and the filtrate, after 6 h, was examined by n.m.r. spectroscopy. Two signals were observed for H-6: τ 1·95 and 1·99 [total 1H, ratio 4 : 7, both $J_{6,5}$ 8·5 Hz, H-6 of the two isomers (XII) and (XIII)].

Hydrogenolysis of the 2′-substituted protected aminoacyl derivative (X) as in (*a*) similarly gave the aforementioned monoacetate in 91% yield (Found: C, 47·2; H, 6·4; N, 10·1%).

Hydrogenolysis of 3′,5′-*Di*-O-*benzyl*-2′-O-(N-*benzyloxycarbonyl*-L-*valyl*)*uridine under Aprotic Conditions*.—A solution of the protected aminoacyl derivative (X) (0·330 g) in anhydrous dioxan (1·5 ml) containing 5% palladium–charcoal (0·19 g) was stirred under hydrogen ($CaCl_2$ guard tube between reaction flask and burette). Reaction occurred slowly, requiring 3 weeks for completion. The product, R_F (dioxan) 0·30, was ninhydrin-positive, but showed very weak u.v. absorption (t.l.c.). No valine was detected. The n.m.r. spectrum of the filtered mixture showed no pyrimidine H-6 signal. Addition of aqueous 4N-ammonia (2 or 3 drops) to 0·2 ml of the reaction mixture, and subsequent examination (t.l.c.) showed the presence of valine in the hydrolysate. A concentrate of a sample of this hydrolysate was inserted into the source of the mass spectrometer, and the source temperature, initially 100°, was raised gradually to 200°. The spectrum recorded at the latter temperature was identical with that of an authentic sample (Sigma Chemical Co.) of dihydrouridine. Diagnostic peaks (*m/e* 73 ≡ 100%) were *m/e* 228 ($M - H_2O$, 4%), 143 (90), and 115 (15).

3′,5′-*Di*-O-*benzyl*-2′-O-*pivaloyluridine* (XIV).—A solution of 3′,5′-di-*O*-benzyluridine (I) (0·300 g, 0·71 mmol) and pivaloyl chloride (0·17 ml, 1·42 mmol) in dry pyridine (6 ml) was stirred at 70° for 2 h, then concentrated. A solution of the residue in toluene was concentrated (×2) to remove residual pyridine prior to chromatography on a column of silicic acid (50 g), which was eluted with ether. The first component eluted was 3′,5′-di-*O*-benzyl-2′-*O*,3-*N*-dipivaloyluridine, a yellow gum, R_F (ether) 0·66 (0·100 g, 24%). This was characterized by n.m.r. spectroscopy and mass spectrometry the elemental analysis being unsatisfactory: τ 2·16 (1H, d, $J_{6,5}$ 9·4 Hz, H-6), 2·63

[19] A. M. Michelson and Sir Alexander Todd, *J. Chem. Soc.*, 1956, 3459.

(10H, m, benzyl aromatic H), 3·90 (1H, d, $J_{1',2'}$ 2·5 Hz, H-1'), 4·58—4·76 (2H, m. H-5 and H-2'), 5·53 (4H, s, benzyl CH_2), and 8·70 and 8·80 (both 9H, s, pivaloyl CH_3), M^+ 592.

The second component eluted was the required 2'-O-*pivaloyl derivative*, R_F (ether) 0·46, obtained, after concentration of the eluate, as white platelets (0·252 g, 70%), m.p. 47—48° (Found: C, 65·9; H, 6·3; N, 5·6. $C_{28}H_{32}N_2O_7$ requires C, 66·1; H, 6·3; N, 5·5%), τ 0·78 (1H, s, H-3), 2·20 (1H, d, $J_{6,5}$ 8·5 Hz, H-6), 2·65 (10H, m, benzyl aromatic H), 3·90 (1H, d, $J_{1',2'}$ 2·5 Hz, H-1'), 4·50—4·80 (2H, m, H-5 and H-2'), 5·53 (4H, s, benzyl CH_2), and 8·80 (9H, s, pivaloyl CH_3), M^+ 508.

2',5'-*Di*-O-*benzyl*-3'-O-*pivaloyluridine* (XV).—2',5'-Di-*O*-benzyluridine (II) (0·200 g, 0·47 mmol) was similarly treated with pivaloyl chloride. The corresponding di-pivaloyl derivative, 2',5'-di-*O*-benzyl-3-*N*,3'-*O*-dipivaloyluridine, a yellow gum, R_F (ether) 0·69 (0·072 g, 26%) was again eluted first: τ 2·74 (11H, m, benzyl aromatic protons + H-6) 3·94 (1H, $J_{1',2'}$ 6·0 Hz, H-1'), 5·50 (4H, s, benzyl CH_2), and 8·70 and 8·80 (both 9H, s, pivaloyl CH_3), M^+ 592.

The required 3'-O-*pivaloyl derivative*, R_F (ether) 0·47 was obtained as white platelets (0·168 g, 71%), m.p. 49—50° (Found: C, 66·6; H, 6·5; N, 5·6%), τ 0·81 (1H, s, H-3), 2·68 (1H, m, benzyl aromatic H + H-6), 3·84 (1H, d, $J_{1',2'}$ 6·0 Hz, H-1'), 4·60—4·80 (2H, m, H-5 + H-3'), 5·48 (4H, s, benzyl CH_2), and 8·76 (9H, s, pivaloyl CH_3), M^+ 508.

2'-O-*Pivaloyluridine* (XVI).—A solution of 3',5'-di-*O*-benzyl-2'-*O*-pivaloyluridine (XIV) (0·185 g, 0·37 mmol), in dry dioxan (5 ml) was hydrogenated over 5% palladium–charcoal (0·10 g) for 7 days. Concentration of the filtered solution (finally at 0·5 mmHg) gave the *product* (0·112 g, 93%), R_F (ethyl acetate) 0·25, a waxy solid of indefinite m.p. (Found: C, 50·8; H, 6·4; N, 8·1. $C_{14}H_{20}N_2O_7$ requires C, 51·2; H, 6·1; N, 8·5%), τ (CH_3·OD) 1·95 (1H, d, $J_{6,5}$ 8·0 Hz, H-6), 3·80—4·10 (2H, m, H-1' and H-2'), 4·25 (1H, d, H-5), and 8·76 (9H, s, pivaloyl CH_3).

3'-O-*Pivaloyluridine* (XVII).—Hydrogenation of 2',5'-di-*O*-benzyl-3'-*O*-pivaloyluridine (XV) (0·106 g, 0·21 mmol), similarly gave the 3'-*analogue* (0·062 g, 91%), a white solid, m.p. 110° (Found: C, 50·7; H, 6·4; N, 8·1%), τ (CH_3·OD), 1·98 (1H, d, $J_{6,5}$ 7·0 Hz, H-6), 4·01 (1H, d, $J_{1',2'}$ 5·0 Hz, H-1'), 4·22 (1H, d, H-5), and 8·71 (9H, s, pivaloyl CH_3).

Migratory Studies on 2'- and 3'-O-Pivaloyluridine.—Morpholine (0·0015 g) was added to a solution of 2'-*O*-pivaloyluridine (XVI) (0·100 g) in CH_3·OD (0·5 ml) and changes in the solution, held at 50°, were monitored by n.m.r. spectroscopy. The relative proportions of the 2'-pivaloyl derivative and its 3'-isomer (XVII) were estimated by measuring the heights of the signals (see before) for the pivaloyl CH_3 groups. The derived ratios 3'-pivaloyl to 2'-pivaloyl, were: at 2 h, 0·33; at 21 h, 0·99; at 40 h, 1·13; at 61 h, 1·27; at 130 h, 1·37. Thereafter, no further change in the ratio was observed. A solution containing initially 3'-*O*-pivaloyluridine gave the same ultimate ratio between the two signals. The equilibration rate constant ($k_1 + k_2$) was determined graphically from the foregoing values using the method of Frost and Pearson [20] as applied by Griffin *et al.*[14] to acyl migration studies. This gave $k_1 + k_2$ as 0·069 h^{-1} (2'-pivaloyluridine $\underset{k_2}{\overset{k_1}{\rightleftharpoons}}$ 3'-pivaloyluridine), and $t_{\frac{1}{2}}[= 0{\cdot}69/(k_1 + k_2)]$ as 10·0 h.

No uridine was formed (t.l.c. monitoring) during the period required for equilibration in either experiment.

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[20] A. A. Frost and R. G. Pearson, 'Kinetics and Mechanism,' Wiley, New York, 1953, p. 172.