*Tetrahedron* Vol. 43, No. 20, pp. 4601 to 4608, 1987
Printed in Great Britain.

0040-4020/87 $3.00+.00


# THE SYNTHESIS AND ANTIVIRAL PROPERTIES OF (E)-5-(2-BROMOVINYL)-2'-DEOXYURIDINE-RELATED COMPOUNDS

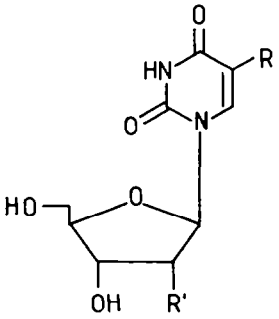
MARK ASHWELL, A. STANLEY JONES,* AJIT KUMAR, JON R. SAYERS, RICHARD T. WALKER, TAKASHI SAKUMA† and ERIK DE CLERCQ†

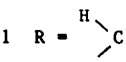
Chemistry Department, The University of Birmingham, Birmingham B15 2TT, Great Britain and †Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

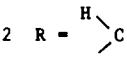
(*Received in UK* 3 *July* 1987)

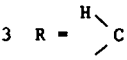
Abstract - A method for the synthesis of the potent anti-herpes agent, (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) (1) is described. A trace bye-product of the synthesis has been identified as 5-(1,2-dibromo-2-succinimidoethyl)-2'-deoxyuridine (5). A similar bye-product, 5-(1,2-dibromo-2-succinimidoethyl)uridine (4) is formed during the synthesis of (E)-5-(2-bromovinyl)uridine (3). The following derivatives of BVDU have been synthesised : 3'-O-methyl (8), 3-methyl (9), 4-O-ethyl (11), $O^2$,5'-anhydro-(E)-5-(2-bromovinyl)-2'-deoxyuridine (13) and (E)-5-(2-bromovinyl)-2'-deoxyisocytidine (15). Whereas compounds 8, 9 and 15 showed little, if any, antiviral activity, 11 and, in particular, 13, were significantly active against herpes simplex virus type 1 and varicella-zoster virus.

(E)-5-(2-Bromovinyl)-2'-deoxyuridine (BVDU) (1) is an extremely potent antiviral agent against herpes simplex virus type 1 (HSV-1) and varicella-zoster virus (VZV).[1] The synthesis and antiviral activity of this compound was first briefly reported by us in 1978.[2] The method used for the synthesis was to condense (E)-5-(2-bromovinyl)uracil[3] with 3,5-di-O-toluoyl-2-deoxy-α-D-ribofuranosyl chloride, separation of the resulting mixture of α and β anomers and removal of the protecting groups. This synthesis has since been described in detail.[4] Because of its tedious nature and low yields, this method is not suitable for the large-scale synthesis of BVDU, so alternative procedures were investigated. The first of these involved the conversion of 2'-deoxyuridine into (E)-5-(2-carboxyvinyl)-2'-deoxyuridine (2) via the 5-chloromercuric derivative and then a 5-palladium derivative. Treatment of 2 with N-bromosuccinimide gave the product.[5] Although this method was quite convenient it was too expensive for commercial use. A synthesis which is suitable for large scale preparation has been developed in collaboration with others[6] and is described in detail in this paper. In this procedure compound 2 is obtained from 2'-deoxy-5-iodouridine by a palladium-catalysed coupling reaction with methylacrylate and subsequent hydrolysis of the ester. Conversion of 2 into the product (1) was achieved with N-bromosuccinimide essentially as previously described.[5] A similar series of reactions has been used to synthesise (E)-5-(2-bromovinyl)uridine (3).[7]



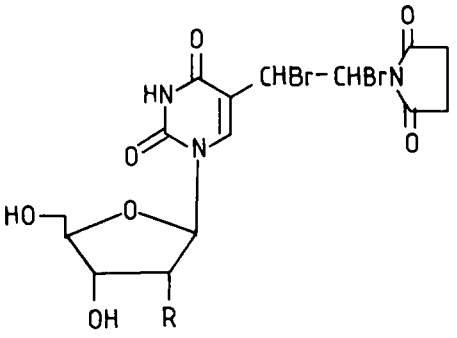
1 R =  ; R' = H

2 R =  ; R' = H

3 R =  ; R' = OH

We have now carried out further studies on this synthetic procedure and also have made derivatives of BVDU (2).

During the final stage of the synthesis of 3 it was noticed that a small amount (~ 3 %) of another nucleoside had been formed. The u.v. spectrum of this minor reaction product suggested that the side chain at C-5 was saturated. On the basis of microanalytical and n.m.r. spectral data it was concluded that the compound was 5-(1,2-dibromo-2-succinimidoethyl)uridine (4). Similar treatment of 2 with N-bromosuccinimide gave in addition to BVDU (1), about 3 % of a product, which from $^{1}H$ n.m.r. spectroscopy, elemental analysis and f.a.b. mass spectrometry was shown to be a 5-(dibromosuccinimidoethyl)-2'-deoxyuridine. The structure, 5-(1,2-dibromo-2-succinimidoethyl)-2'-deoxyuridine (5) was assigned by analogy with 4. It was expected that 4 and 5 would be mixtures of diastereoisomers, but the signals in the $^{1}H$ and $^{13}C$ n.m.r. spectra were not resolved.

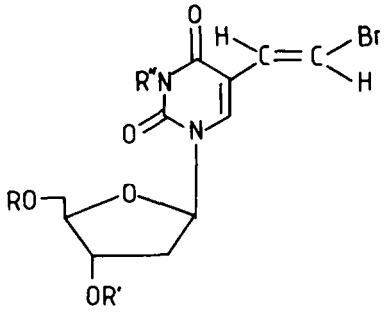



4 R = OH
5 R = H

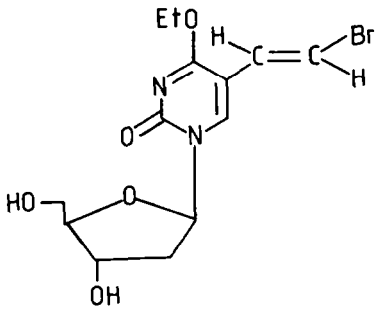
As it was thought that 4 and 5 might have arisen by the addition of N-bromosuccinimide to 3 and 1 respectively, 1 was treated with one equivalent of N-bromosuccinimide under the appropriate conditions. A complex mixture of products (> 6) was obtained which was only partly separable by column chromatography. Two of these products had f.a.b. mass spectra which were consistent with structure 5. However, this result makes it doubtful whether 5 was formed from 1. It appears more likely that 5 arose from 2 by an alternative pathway. The presence of 5 as an impurity in BVDU might have some consequences for the clinical use of the latter.

In order to study the effect of substituents on the biological activity of BVDU, some alkyl derivatives have been synthesized. Treatment of 1 with t-butyldiphenylsilyl chloride gave the 5'-O-t-butyldiphenylsilyl derivative (6) in 67 % yield. Reaction of 6 with methyl iodide and sodium hydride (2 equivalents) in tetrahydrofuran gave the 3'-O-methyl derivative (7). The structure assigned to 7 was based upon the n.m.r. spectrum which showed the presence of an -NH and an $-OCH_3$ group and the absence of a 3'-OH group. The u.v. spectrum also showed the presence of an -NH group. Treatment of 7 with tetrabutylammonium fluoride gave (E)-5-(2-bromovinyl)-2'-deoxy-3'-O-methyluridine (8). Reaction of 1 with methyl iodide in methanol in the presence of sodium carbonate gave (E)-5-(2-bromovinyl)-2'-deoxy-3-methyluridine (9) which was identified by its lack of an -NH group and the presence of $-NCH_3$, 3'-OH and 5'-OH groups (n.m.r.)

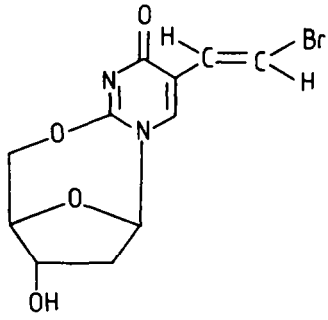
The 4-O-ethyl derivative of BVDU was obtained by treating 3',5'-di-O-acetyl-(E)-5-(2-bromovinyl)-2'-deoxyuridine (10) with phosphoryl chloride and N-methylimidazole in acetonitrile. The intermediate 3-methylimidazolium compound so formed was treated with ethanol and then triethylamine to give (E)-5-(2-bromovinyl)-2'-deoxy-4-O-ethyluridine (11). The synthesis of 4-O-alkyl derivatives of thymidine by a similar procedure has been reported recently.[8] To obtain the 2-O-methyl derivative of BVDU, (E)-5-(2-bromovinyl)-2'-deoxy-5'-O-p-toluenesulphonyluridine (12) was treated with base to give $O^{2}$,5'-anhydro-(E)-5-(2-bromovinyl)-2'-deoxyuridine (13), which upon treatment with methanol gave (E)-5-(2-bromovinyl)-2'-deoxy-2-O-methyluridine (14). A similar series of reactions, but using ammonia in methanol to open the anhydro ring of 13 gave (E)-5-(2-bromovinyl)-2'-deoxyisocytidine (15).



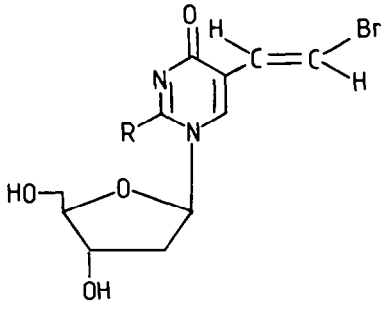
6 R = t-butyldiphenylsilyl; R',R" = H
7 R = t-butyldiphenylsilyl; R' = $-CH_3$; R" = H
8 R,R" = H; R' = $CH_3$
9 R,R' = H, R" = $CH_3$
10 R,R' = $COCH_3$ ; R" = H
12 R = p-toluenesulphonyl; R',R" = H



11



13



14 R = $-OCH_3$
15 R = $-NH_2$

As has already been reported,[9] compound 10 shows a significant activity against herpes simplex virus type 1 ($MIC_{50}$ 0.2 μg/ml). The activity has been confirmed by the present investigations (Table 1). Compound 8 has no antiviral activity and compound 9 has low activity against HSV-1 (Table 1). This extends previously reported data for compounds 8 and 9[10] and also points to the necessity of both the 3'-OH and the 3-NH groups of the high activity of BVDU. Compound 11 was 100-fold, and compound 13 was only 10-fold less active against HSV-1 than was BVDU (Table 1). With BVDU, compounds 11 and 13 also shared a similar activity spectrum, in that they were considerably less active against HSV-2 and vaccinia virus and inactive against the RNA (vesicular stomatitis) virus and thymidine kinase-deficient ($TK^-$) HSV-1 variants. Compound 15 did not show an appreciable antiviral effect (Table 1).

Because of their marked activity against HSV-1, compounds 11 and 13 were further evaluated against a broader array of herpesviruses, and as shown in Table 2, they proved active against VZV at the same $MIC_{50}$'s as found for their activity against HSV-1. They were also inhibitory to suid herpesvirus type 1 (SHV-1), bovid herpesvirus type 1 (BHV-1) and herpesvirus platyrrhinae (HVP), but not to equid herpesvirus type 1 (EHV-1) or $TK^-$ VZV variants.

Table 1. Antiviral activity of compounds 1, 8, 9, 10, 11, 13 and 15 against herpes simplex virus

| Virus | Minimum inhibitory concentration ($MIC_{50}$)* (μg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 8 | 9 | 10 | 11 | 13 | 15 | 1 (BVDU) |
| HSV-1 (KOS) | > 400 | 20 | 0.2 | 2 | 0.2 | 70 | 0.02 |
| HSV-1 (F) | > 400 | 70 | 0.2 | 2 | 0.2 | 70 | 0.02 |
| HSV-1 (McIntyre) | > 400 | 20 | 0.2 | 2 | 0.4 | 150 | 0.02 |
| HSV-2 (G) | 300 | 150 | 20 | 150 | 40 | > 400 | 2 |
| HSV-2 (196) | 300 | 150 | 70 | 300 | 150 | > 400 | 20 |
| HSV-2 (Lyons) | > 400 | 150 | 70 | 150 | 30 | > 400 | 7 |
| Vaccinia | > 400 | > 200 | 7 | 70 | 40 | > 400 | 7 |
| Vesicular stomatitis | > 400 | > 400 | > 400 | > 400 | > 400 | > 400 | > 400 |
| $TK^-$ HSV-1 (B2006) | ND | ND | ND | 300 | 300 | > 400 | 100 |
| $TK^-$ HSV-1 (VMW 1837) | ND | ND | ND | 300 | 300 | > 400 | > 400 |

* Required to reduce virus-induced cytopathogenicity in primary rabbit kidney cell cultures by 50 %. For further details on the virus strains and methods used, see ref. 11.
ND : not determined.

Table 2. Antiviral activity of compounds 1, 11 and 13 against varicella-zoster virus and other herpesviruses

| Compound | Minimum inhibitory concentration ($MIC_{50}$)* (μg/ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | VZV | | | | CMV | | SHV-1 | BHV-1 | HVP | EHV-1 |
| | YS | Oka | YSR ($TK^-$) | 07-1 ($TK^-$) | Davis | AD-169 | | | | |
| 11 | 2 | 2.5 | > 400 | > 400 | 20 | 20 | 2 | 2 | 2 | 300 |
| 13 | 0.1 | 0.2 | > 400 | > 400 | > 100 | > 100 | 0.7 | 2 | 0.2 | > 400 |
| 1 (BVDU) | 0.006 | 0.007 | > 400 | > 400 | 300 | 200 | 0.07 | 0.2 | 0.02 | > 400 |

* Required to reduce virus-induced cytopathogenicity in primary rabbit kidney cells (SHV-1, BHV-1, HVP, EHV-1) or human embryonic lung cells (VZV, CMV) by 50 %. For further details on the virus strains and methods used, see ref. 15. Data for 1 were taken from ref. 11.

From the data presented in this report it thus appears that the presence of a 4-O-ethyl group and $O^2$,5'-anhydro linkage in BVDU are compatible with significant antiviral activity, and the activity spectrum of the products (11 and 13) is remarkably similar to that of BVDU.[12]

## EXPERIMENTAL

N.m.r. spectra were recorded on Varian XL100 (100 MHz), Jeol FX90Q (90 MHz) and Jeol GX270 (270 MHz) instruments with $(CD_3)_2SO$ as solvent unless otherwise stated. U.v. spectra were measured on a Perkin-Elmer 552 spectrophotometer and mass spectra on a Kratos MS80 mass spectrometer. Column chromatography was carried out on silica gel, Kieselgel 60 type 7734, 0.063-0.200 mm, 70-230 mesh ASTM (E. Merck A.G., Darmstadt, W. Germany). All experiments were carried out under scrupulously dry conditions unless otherwise stated and all evaporations were carried out under reduced pressure.

(E)-5-(2-Carbomethoxyvinyl)-2'-deoxyuridine. A mixture of palladium (II) acetate (0.21 g, 0.9 mmole), triphenylphosphine (0.52 g, 1.9 mmole) and redistilled triethylamine (3.5 ml) in dioxane (35 ml) was stirred at 70°C until an intense red colour had developed. To this there was then added 2'-deoxy-5-iodouridine (7.0 g, 1.9 mmole) and methyl acrylate (3.3 g, 3.8 mmole) as a slurry in dioxan (10 ml) and the mixture boiled under reflux for 40 min. It was then filtered while still hot and the filtrate cooled overnight to 4°C. The resulting pale-yellow precipitate was filtered off, washed with dichloromethane (3 x 15 ml) and dried in vacuo to give the product as a white solid (4.2 g, 70 % yield), m.p. 170°C; u.v. (pH 7 in $H_2O$) $\lambda_{max}$ 269 nm (sh) (ε, 10900), 301 nm (ε, 16700), $\lambda_{min}$ 271 nm (ε, 10800); n.m.r. δ 2.2 (2H, m, H-2'), 3.6 (2H, m, H-5'), 3.7 (3H, s, $-CH_3$), 3.8 (1H, m, H-4'), 4.31 (1H, m, H-3'), 5.2 (2H, bs, -OH-3', -OH-5'), 6.12 (1H, t, H-1'), 6.83 (1H, d, vinylic H, J 16 Hz), 7.37 (1H, d, vinylic H, J 16 Hz), 8.41 (1H, s, H-6), 11.0 (1H, bs, -NH).

(E)-5-(2-Carboxyvinyl)-2'-deoxyuridine (2). (E)-5-(2-Carbomethoxyvinyl)-2'-deoxyuridine (2.0 g, 6.4 mmole) was added to aqueous 1M NaOH (100 ml) and the mixture stirred at ∿ 20°C for 40 h, filtered and the clear filtrate adjusted to pH 2 with 1M HCl. On cooling to ∿ 4°C a white precipitate formed. This was filtered off and washed with water (2 x 20 ml) and acetone (2 x 20 ml) and dried to give the product (1.3 g, 65 % yield), m.p. 267°C; u.v. (pH 7 in $H_2O$) $\lambda_{max}$ 263 nm (ε, 11800), 297 nm (ε, 14600), $\lambda_{min}$ 275 nm (ε, 10900); n.m.r. 2.18 (2H, t, H-2'), 3.62 (2H, m, H-5'), 3.8 (1H, m, H-5'), 4.22 (1H, m, H-3'), 5.15 (2H, bs, -OH-3', -OH-5'), 6.11 (1H, t, H-1'), 6.74 (1H, d, vinylic H, J 16 Hz), 7.25 (1H, d, vinylic H, J 16 Hz), 8.35 (1H, s, H-6), 10.9 (1H, bs, $-CO_2H$), 11.4 (1H, bs, -NH).

(E)-5-(2-Bromovinyl)-2'-deoxyuridine (1). To a solution of compound 2 (5.0 g, 15 mmole) in dimethylformamide (DMF) (25 ml) there was added potassium carbonate (4.75 g, 34 mmole) and the suspension stirred at ∿ 20°C for 15 min. A solution of N-bromosuccinimide (2.84 g, 16 mmole) in DMF (25 ml) was added dropwise over 30 min at ∿ 20°C. The resulting suspension was filtered immediately under suction and the solid washed well with DMF. The combined filtrate and washings were evaporated to dryness in vacuo to complete remove the DMF and the residue dissolved in ethanol. To this silica gel was added and the suspension evaporated to dryness and the solid applied to the top of a silica gel column prepared in chloroform-ethanol (9:1). The column was eluted with the same solvent followed by chloroform-ethanol (3:1). The first nucleoside eluted was the product (3.79 g, 68 % yield). Crystallisation from water gave white needles, m.p. 141°C (Found: C, 39.4; H, 4.0; N, 8.4. Calc. for $C_{11}H_{13}BrN_2O_5$ C, 39.7; H, 3.9; N, 4.1 %); u.v. (ethanol) $\lambda_{max}$ 250 nm (ε, 13100), 294 nm (ε, 10250), $\lambda_{min}$ 273 nm (ε, 6400); n.m.r. δ 2.15 (2H, m, H-2'), 3.60 (2H, m, H-2'), 3.60 (2H, m, H-5'), 3.80 (1H, m, H-4'), 4.25 (1H, m, H-3'), 5.07 (1H, t, -OH-5'), 5.23 (1H, d, -OH-3'), 6.13 (1H, t, H-1'), 6.82 (1H, d, vinylic H, J 14 Hz), 7.26 (1H, d, vinylic H, J 14 Hz), 8.08 (1H, s, H-6), 11.57 (1H, s, -NH).

5-(1,2-Dibromo-2-succinimidoethyl)-2'-deoxyuridine (5). Further elution of the column used to isolate 1 gave another nucleoside product (150 mg, 3 % yield) which was identified as 5 (Found : C, 35.5; H, 3.2; N, 7.9. $C_{15}H_{17}Br_2N_3O_7$ requires C, 35.25; H, 3.35; N, 8.2 %); u.v. (ethanol) $\lambda_{max}$ 267 nm (ε, 10600); n.m.r. δ 1.95-2.20 (2H, m, H-2'), 2.70 (4H, s, $-CH_2-CH_2-$), 3.60 (1H, m, H-5'), 3.80 (1H, m, H-4'), 4.30 (1H, bd, H-3'), 5.10 (1H, t, -OH-5'), 5.25 (1H, d, -OH-3'), 5.65 (1H, d, -CHBr-N-), 6.20 (1H, t, H-1'), 6.60 (1H, d, -CHBr-C=), 8.40 (1H, s, H-6), 11.55 (1H, s, -NH); f.a.b. mass spec. m/z 512 (M + H), 396 (Base + H)$^+$, 297 (Base - succinimide + H)$^+$. All peaks showed isotopic satellites in the ratio 1:2:1.

5-(1,2-Dibromo-2-succinimidoethyl)uridine (4). (E)-5-(2-Carboxyvinyl)uridine (8.64 g)[7] was treated with N-bromosuccinimide under the same conditions as described above for compound 2 and the reaction mixture worked up in a similar way. The first nucleoside eluted from the column was (E)-5-(2-bromovinyl)uridine (5.9 g, 62 % yield), identical to that previously obtained by us.[7]
Further elution of the column gave the product (4) (250 mg, ∿ 3 % yield) (Found : C, 34.1; H, 3.2; N, 8.0; $C_{15}H_{17}Br_2N_3O_8$ requires C, 34.2; H, 3.25; N, 8.0 %); u.v. (ethanol) $\lambda_{max}$ 267 nm (ε, 10580); $^1$H n.m.r. δ 2.70 (4H, s, $-CH_2-CH_2-$), 3.55-3.75 (2H, m, H-5'), 3.80-3.90 (1H, m, H-4'), 3.90-4.20 (2H, m, H-2', H-3'), 5.00-5.40 (3H, bd, -OH-2', -OH-3', -OH-5'), 5.70 (1H, d, -CHBr-N-), 5.85 (1H, d, H-1'), 6.65 (1H, d, -CHBr-C=), 8.40 (1H, s, H-6), 11.60 (1H, bd, -NH); f.a.b. mass spec. m/z 528 (M + H)$^+$, 396 (Base + H)$^+$, 297 (Base - succinimide + H)$^+$. All peaks show isotopic satellites in the ratio 1:2:1. $^{13}$C n.m.r. 27.5 ($-CH_2-CH_2$), 44.1 [$-C(Br)N(COCH_2)_2$], 53.0 (-CHBr), 61.2 (C-5'), 70.1 (C-2'), 73.9 (C-3'), 85.2 (C-5'), 88.3 (C-1'), 106.7 (C-5), 142.1 (C-6), 149.8 (C-2), 161.8 (C-4), 176.5 (2 x C=O). The "Inept" and "off resonance" procedures were carried out as described in the literature.[13,14]

(E)-5-(2-Bromovinyl)-2'-deoxy-5'-O-t-butyldiphenylsilyluridine (6). A mixture of compound 1 (1.56 g, 4.7 mmole), triethylamine (0.52 g, 5.1 mmole), dimethylaminopyridine (0.15 g, 1.2 mmole) and t-butyldiphenylsilyl chloride (1.43 g, 5.2 mmole) in DMF (20 ml) was stirred at ∿ 20°C for 12 h. Water (10 ml) was then added and the mixture evaporated to leave a gum which was dissolved in chloroform (50 ml). This solution was extracted with water (2 x 30 ml) and the chloroform layer

separated and evaporated to a small volume and the material purified by column chromatography. Elution of the column with chloroform and evaporation of the appropriate fractions gave the required material as a white solid. Crystallisation from chloroform–hexane gave the product as fine white needles (1.8 g, 67 % yield) (Found : C, 57.0; H, 5.5.; N, 4.8. $C_{27}H_{31}BrN_2O_5Si$ requires C, 56.7; H, 5.5; N, 4.9 %); u.v. (pH 7 in $H_2O$) $\lambda_{max}$ 247 nm (ε, 11780), 293 nm (ε, 10090), 232 nm (ε, 9940), 270 nm (ε, 6470); (alkaline ethanol) $\lambda_{max}$ 240 nm (ε, 13310), 287 nm (ε, 9060), $\lambda_{min}$ 235 nm (ε, 11530), 252 nm (ε, 8870); n.m.r. δ 1.02 (9H, s, $SiC(CH_3)_3$), 2.20 (2H, t, H-2'), 3.87 (3H, m, H-4, H-5'), 4.30 (1H, m, H-3'), 5.30 (1H, s, -OH-3'), 6.18 (1H, t, H-1'), 6.56 (1H, d, vinylic H, J 16 Hz), 7.22 (1H, d, vinylic H, J 15 Hz), 7.35 (5H, m, ArH), 7.50–7.75 (6H, m, ArH, H-6), 11.6 (1H, s, -NH).

(E)-5-(2-Bromovinyl)-2'-deoxy-3'-O-methyl-5'-O-t-butyldiphenylsilyluridine (7). Compound 6 (1.02 g, 1.8 mmole) was suspended in tetrahydrofuran (20 ml) and sodium hydride (87 mg, 3.9 mmole) was added and the mixture stirred for a further 6 h. It was then poured into ethanol (40 ml), neutralised with glacial acetic acid and evaporated to dryness. The residue was fractionated by column chromatography using chloroform–methanol (19:1) as the eluant. Removal of the solvent from the appropriate fractions gave the product as a white solid (400 mg, 40 % yield) (Found : C, 57.5; H, 5.7; N, 5.0. $C_{28}H_{33}BrN_2O_5Si$ requires C, 57.2; H, 5.7; N, 4.8 %); u.v. (pH 7 in $H_2O$) $\lambda_{max}$ 247 nm (ε, 13510), 292 nm (ε, 11490), $\lambda_{min}$ 270 nm (ε, 7432); (alkaline ethanol) $\lambda_{max}$ 255 nm (ε, 10560), $\lambda_{min}$ 272 (ε, 10180); n.m.r. δ 1.04 (9H, s, $-Si(CH_3)_3$), 2.30 (2H, m, H-2'), 3.28 (3H, s, $-OCH_3$), 3.87 (2H, m, H-5'), 4.03 (2H, m, H-3', H-4'), 6.10 (1H, t, H-1'), 6.60 (1H, d, vinylic H, J 15 Hz), 7.25 (1H, d, vinylic H, J 15 Hz), 7.42 (5H, m, ArH), 7.61 (5H, m, ArH), 7.72 (1H, s, H-6), 11.62 (1H, s, -NH).

(E)-5-(2-Bromovinyl)-2'-deoxy-3'-O-methyluridine (8). A mixture of compound 7 (347 mg, 0.6 mmole) and tetrabutylammonium fluoride trihydrate (270 mg, 0.8 mmole) in tetrahydrofuran (30 ml) was stirred at 20°C for 18 h. Ethanol (10 ml) was then added and the mixture evaporated to dryness. The residue was fractionated by column chromatography. Elution of the column with chloroform–ethanol (19:1) and evaporation of the appropriate fractions gave the product as a white solid (150 mg, 70 % yield) (Found : C, 41.5; H, 4.4; N, 7.9. $C_{12}H_{15}BrN_2O_5$ requires C, 41.5; H, 4.4; N, 8.1 %); u.v. (pH 7 in $H_2O$) $\lambda_{max}$ 247 nm (ε, 14380), 293 nm (ε, 12218), $\lambda_{min}$ 267 nm (ε, 7660); (alkaline ethanol) $\lambda_{max}$ 253 nm (ε, 15170), 285 nm (ε, 10890), $\lambda_{min}$ 274 nm (ε, 10314); n.m.r. δ 2.24 (2H, m, H-2'), 3.28 (3H, s, $-OCH_3$), 3.60 (2H, m, H-5'), 3.95 (2H, m, H-3', H-5'), 5.13 (1H, t, -OH-5'), 6.06 (1H, t, H-1'), 6.83 (1H, d, vinylic H, J 15 Hz), 7.25 (1H, d, vinylic H, J 15 Hz), 8.04 (1H, s, H-6), 11.58 (1H, s, -NH).

(E)-5-(2-Bromovinyl)-2'-deoxy-3-methyluridine (9). A solution of compound 1 (400 mg, 1.2 mmole) in methanol (30 ml) was stirred with sodium carbonate (260 mg, 2.4 mmole). Methyl iodide (270 mg, 1.9 mmole) was added, the vessel sealed and the mixture stirred at ∿20°C for 18 h. The reaction mixture was evaporated to dryness and the residue extracted with acetone. The acetone extract was filtered, the filtrate evaporated to dryness and the residue fractionated by column chromatography. The column was eluted with chloroform–methanol (19:1) and the appropriate fractions collected and evaporated to dryness to give a white solid which was crystallised from water to give the product (200 mg, 47 % yield) (Found : C, 41.8; H, 4.2; N, 7.9. $C_{12}H_{15}BrN_2O_5$ requires C, 41.5; H, 4.4; N, 8.1 %); u.v. (ethanol) $\lambda_{max}$ 248 nm (ε, 15300), 292 nm (ε, 12100), $\lambda_{min}$ 268 nm (ε, 7930); n.m.r. δ 2.14 (2H, m, H-2'), 3.18 (3H, s, $-NCH_3$), 3.61 (2H, m, H-5'), 3.80 (1H, m, H-4'), 4.22 (1H, m, H-3'), 5.10 (1H, t, -OH-5'), 5.24 (1H, d, -OH-3'), 6.15 (1H, t, H-1'), 6.84 (1H, d, vinylic H, J 16 Hz), 7.27 (1H, d, vinylic H, J 16 Hz), 7.15 (1H, s, H-6).

3',5'-Di-O-acetyl(E)-5-(2-bromovinyl)-2'-deoxyuridine (10). A mixture of compound 1 (4.0 g, 12 mmole), pyridine (50 ml) and acetic anhydride (5.5 ml, 63 mmole) was stirred at ∿20°C for 14 h. The mixture was then co-evaporated with ethanol (3 x 20 ml) and then with toluene (4 x 20 ml) to give a white solid which was purified by column chromatography. Elution of the column with chloroform and evaporation of the appropriate fractions gave a white solid which was crystallised from propan-2-ol to give the product as white needles (3.89 g, 70 % yield), m.p. 156°C (Found: C, 43.5; H, 4.4; N, 6.7. $C_{15}H_{17}BrN_2O_7$ requires C, 43.2; H, 4.1; N, 6.7 %); u.v. (pH 12 in $H_2O$) $\lambda_{max}$ 256 nm (ε, 14420), 280 nm (ε, 9300), $\lambda_{min}$ 275 nm (ε, 9270); n.m.r. δ 2.06 (3H, s, $-COCH_3$), 2.08 (3H, s, $COCH_3$), 2.45 (2H, m, H-2'), 4.24 (3H, m, H-4', H-5'), 5.21 (1H, m, H-3), 6.17 (1H, t, H-1'), 6.78 (1H, d, vinylic H, J 15 Hz), 7.30 (1H, d, vinylic H, J 15 Hz), 7.83 (1H, s, H-6), 11.64 (1H, s, -NH).

(E)-5-(2-Bromovinyl)-2'-deoxy-4-O-ethyluridine (11). To a mixture of freshly-distilled phosphoryl chloride (3.3 g, 22 mmole) and acetonitrile (50 ml) at 0°C, there was added, with stirring, N-methylimidazole (5.9 g, 70 mmole). The solution turned yellow and a solid precipitated. To this suspension compound 10 (3.0 g, 7.2 mmole) was added and then after 2 h at 20°C there was added a solution of triethylamine (1 ml) in ethanol (10 ml). The mixture was stirred at 20°C for 18 h and then evaporated to dryness. The residue was suspended in a mixture of methanol and aqueous ammonia (sp.g. 0.88) (5:1, 50 ml), heated at 50°C for 1 h, evaporated to dryness and triturated with ether (20 ml) and chloroform (20 ml). The solid so obtained was crystallised from aqueous ethanol to give the product as colourless needles (1.6 g, 65 % yield) (Found : C, 42.9; H, 4.5; N, 7.5. $C_{13}H_{17}BrN_2O_5$ requires C, 43.2; H, 4.7; N, 7.8 %); u.v. (ethanol) $\lambda_{max}$ 259 nm (ε, 17800), 296 nm (ε, 5000), $\lambda_{min}$ 283 nm (ε, 4700); n.m.r. δ 1.34 (3H, t, $-CH_3$), 2.2 (2H, m, H-2'), 3.65 (2H, m, H-5'), 3.90 (1H, m, H-4'), 4.25 (1H, m, H-3'), 4.40 (2H, q, $-CH_2-$), 5.15 (1H, t, -OH-5'), 5.24 (1H, d, -OH-3'), 6.10 (1H, t, H-1'), 6.93 (2H, s, vinylic H), 8.45 (1H, s, H-6); f.a.b. mass spec. m/z 363 $(M + H)^+$ 15 %, 245 (Base + H)$^+$ 100 %, 117 (sugar)$^+$ 35 %.

(E)-5-(2-Bromovinyl)-2'-deoxy-5'-O-p-toluenesulphonyluridine (12). Compound 1 (10 g, 30 mmole) and p-toluenesulphonyl chloride (6.27 g, 33 mmole) were added to pyridine (120 ml) at 0°C with stirring. The reaction mixture was then kept at 0–5°C for 240 h. Then methanol (50 ml) was added,

the solution evaporated to dryness and the residue crystallised twice from ethanol to give the product (9.2 g, 5.6 % yield) (Found : C, 44.1; H, 3.8; N, 6.0. $C_{18}H_{19}BrN_2O_7S$ requires C, 44.4; H, 3.9; N, 5.8 %); u.v. (ethanol) $\lambda_{max}$ 227 nm (ε, 20650), 250 nm (ε, 14700), 290 nm (ε, 11650), $\lambda_{min}$ 243 nm (ε, 13900), 269 nm (ε, 7750); n.m.r. δ 2.20 (2H, m, H-2'), 2.44 (3H, s, $-CH_3$), 3.90 (1H, m, H-4'), 4.25 (3H, m, H-3', H-5'), 5.50 (1H, bs, -OH-3'), 6.17 (1H, t, H-1'), 6.85 (1H, d, vinylic H, J 16 Hz), 7.30 (1H, d, vinylic H,J 16 Hz), 7.4-7.8 (5H, m, ArH + H-6), 11.60 (1H, s, -NH).

$O^2$,5'-Anhydro-(E)-5-(2-bromovinyl)-2'-deoxyuridine (13). A suspension of compound 12 (620 mg, 1.1 mmole) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (300 mg, 2 mmole) in acetonitrile (30 ml) was boiled under reflux with stirring for 15 min. The mixture was filtered while still hot and the filtrate evaporated to dryness. The residue was purified on a short silica column by elution with chloroform-methanol (8:1). Evaporation of the appropriate fractions gave a residue which was crystallised from water to give the product (100 mg, 29 % yield) (Found : C, 41.6; H, 3.7; N, 8.7. $C_{11}H_{11}N_2O_4Br$ requires C, 41.9; H, 3.5; N, 8.9 %); u.v. $\lambda_{max}$ 210 nm (ε, 19000), 276 nm (ε, 13000), $\lambda_{min}$ 235 nm (ε, 7700); n.m.r. δ 2.30 (2H, m, H-2'), 4.0-4.5 (4H, m, H-3', H-4', H-5'), 5.30 (1H, d, -OH-3'), 6.03 (1H, d, H-1'), 6.86 (1H, d, vinylic H, J 16 Hz), 7.60 (1H, d, vinylic H, J 16 hz), 8.25 (1H, s, H-6).

(E)-5-(2-Bromovinyl)-2'-deoxy-2-O-methyluridine (14). A suspension of compound 12 (3.0 g, 6.2 mmole) and DBU (1.5 g, 9.8 mmole) in acetonitrile (100 ml) was boiled under reflux for 15 min. The solvent was removed by evaporation and to the residue there was added methanol (50 ml) and triethylamine (10 ml) and the mixture boiled under reflux for 2 h. The solvent was removed by evaporation and the residue purified by column chromatography using chloroform-ethanol (7:3) as the eluant. The appropriate fractions were collected and evaporated to dryness to give the product as a white solid (1.0 g, 47 % field) (Found : C, 41.7; H, 4.2; N, 7.8. $C_{12}H_{15}BrN_2O_5$ requires C, 41.5; H, 4.4; N, 8.1 %); u.v. (ethanol) $\lambda_{max}$ 210 nm (ε, 13180), 267 nm (ε, 7400), $\lambda_{min}$ 229 nm (ε, 1550); n.m.r. δ 2.22 (2H, t, H-2'), 3.60 (2H, m, H-5'), 3.83 (1H, m, H-4'), 3.90 (3H, s, $-OCH_3$), 4.25 (1H, m, H-3'), 5.0-5.4 (2H, bm, -OH-3', -OH-5'), 6.10 (1H, t, H-1'), 6.88 (1H, d, vinylic H, J 15 Hz), 7.52 (1H, d, vinylic H, J 15 Hz), 8.81 (1H, s, H-6); f.a.b. mass spec. m/z 347 $(M + H)^+$ 15 %, 231 (Base + H)$^+$ 100 %, 117 (sugar)$^+$ 95 %.

(E)-5-(2-Bromovinyl)-2'-deoxyisocytidine (15). A suspension of compound 12 (1.5 g, 3.1 mmole) and DBU (760 mg, 4.9 mmole) in acetonitrile (100 ml) was boiled under reflux for 20 min. The solvent was then removed by evaporation and to the residue there was added methanol saturated with ammonia (50 ml) and the solution kept at 0-5°C in a sealed vessel for 240 h. The solvent was then removed by evaporation and the residue fractionated by column chromatography using chloroform-methanol (4:1) as the eluant. Evaporation of the solvent from the appropriate fractions gave a solid which was crystallised from methanol to give the product (200 mg, 19 % yield) (Found : C, 39.5; H, 4.0; N, 12.4. $C_{11}H_{14}BrN_3O_4$ requires C, 39.8; H, 4.2; N, 12.7 %); u.v. ($H_2O$ pH 6) $\lambda_{max}$ 261 nm (ε, 14290); 281 nm (sh) (ε, 12590), $\lambda_{min}$ 238 nm; (alkaline ethanol) $\lambda_{max}$ 260 nm (ε, 23650), 298 nm (sh) (ε, 7110), $\lambda_{min}$ 236 nm (ε, 12650); n.m.r. δ 2.22 (2H, m, H-2'), 3.65 (2H, m, H-5'), 3.80 (1H, m, H-5'), 4.30 (1H, m, H-3'), 5.25 (2H, m, -OH-3', -OH-5'), 5.90 (1H, t, H-1'), 6.75 (1H, d, vinylic H, J 16 Hz), 7.15 (2H, bs, $-NH_2$), 7.45 (1H, d, vinylic H, J 16 Hz), 7.93 (1H, s, H-6); f.a.b. mass spec. m/z 332 $(M + H)^+$ 10 %, 216 (Base + H)$^+$ 30 %, 117 (sugar)$^+$ 40 %.

ACKNOWLEDGEMENTS

The authors thank the British Science and Engineering Research Council for research studentships (to M.A. and J.R.S.), Anita Van Lierde for excellent technical help with the antiviral assays and Christiane Callebaut for fine editorial assistance.

REFERENCES

1. E. De Clercq and R.T. Walker, Pharmac. Ther., 26, 1 (1984) and references cited therein.

2. R.T. Walker, P.J. Barr, E. De Clercq, J. Descamps, A.S. Jones and P. Serafiwnoski, Nucleic Acids Research, Spec. Pub. 4, s103 (1978).

3. R.C. Bleackley, A.S. Jones and R.T. Walker, Tetrahedron, 32, 2795 (1976).

4. P.J. Barr, A.S. Jones, G. Verhelst and R.T. Walker, J. Chem. Soc., Perkin Trans. I, 1665 (1981).

5. A.S. Jones, G. Verhelst and R.T. Walker, Tetrahedron Lett., 4415 (1979).

6. R.L. Dyer, A.S. Jones, R.T. Walker, R. Busson and H. Vanderhaeghe, Nucleic Acid Chemistry, Part 4 (Ed. L.B. Townsend and R.S. Tipson), Wiley, in press.

7. E. De Clercq, C. Desgranges, P. Herdewijn, I.S. Sim, A.S. Jones, M.J. McClean and R.T. Walker, J. Med. Chem., 29, 213 (1986).

8. L. Kiriasis, S. Farkas and W. Pfleiderer, Nucleosides and Nucleotides, 5, 517 (1986).

9. R.T. Walker, M.J. Slater, A.S. Jones, J. Balzarini and E. De Clercq, Nucleic Acids Research, Symposium Series, No. 16, 219 (1985).

10. J. Balzarini, E. De Clercq, A. Verbruggen, D. Ayusawa and T. Seno, Mol. Pharmacol., 28, 518 (1985).

11. E. De Clercq, A. Holy, I. Rosenberg, T. Sakuma, J. Balzarini and P.C. Maudgal, Nature, 323, 464 (1986).

12. E. De Clercq, J. Antimicrob. Chemother., 14, suppl. A, 85 (1984).

13. D.M. Doddrell and D.T. Pegg, J. Amer. Chem. Soc., 102, 6388 (1980).

14. R.J. Abraham and P. Lofthus, Proton and Carbon-13 N.M.R. Spectroscopy, Heyden, London and Philadelphia, 1978, pp. 142-143.