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SYNTHESIS OF CYTOSININE, THE NUCLEOSIDE COMPONENT OF ANTIBIOTIC BLASTICIDIN S*

T. KONDO, H. NAKAI and T. GOTO†
Department of Agricultural Chemistry, Nagoya University, Chikusa, Nagoya 464, Japan

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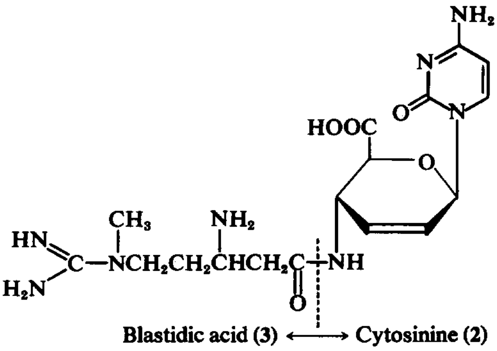
Abstract—The first total synthesis of cytosinine (2) is described.

Blasticidin S (1)[1] is a nucleoside antibiotic produced by *Streptomyces griseochromogenes* and has been used extensively as an excellent fungicide against the virulent fungus, *Piricularia oryzae*, a serious cause of rice blast disease in Japan.[2] It was also reported that the antibiotic inhibits the growth of tobacco mosaic virus[3] and has antitumor activity for certain carcinomas.[4] The structure[5,6] and absolute configuration[7] have been elucidated chemically by Otake *et al* and confirmed by X-ray analysis.[8] Controlled acid hydrolysis of blasticidin S yielded cytosinine (2), a peculiar nucleoside, and blastidic acid (3), a new β-amino acid.[6] In this paper we describe the first synthesis of cytosinine (2).



Blasticidin S (1)

Fig 1.

There seem to be two main procedures for introduction of a 2′,3′-double bond in pyranosyl nucleosides. One involves the elimination of 2′,3′-*trans*-diaxially oriented substituents such as iodosulfonate or disulfonate with iodide and zinc.[9] This method has been applied to the synthetic approach towards cytosinine by Watanabe *et al.*[10] The other method involves direct condensation of a glycal with a base with simultaneous migration of the double bond to the 2′,3′-position. This method has been applied to the synthesis of purine nucleosides,[11] but pyrimidines do not condense with glycals in the usual manner. We found[12] that triacetylglucal could be condensed with silylated uracil in the presence of $SbCl_5$ to afford a mixture of α- and β-anomers of the 2′,3′-unsaturated nucleoside, which could be separated easily by column chromatography. Although this procedure has an intrinsic disadvantage to produce the anomer mixture, we applied it for the synthesis of cytosinine since the reaction is simple and the yield is fairly good.

We have chosen triacetylgalactal (4) and silylated uracil **5**, instead of 4-aminouronic acid and/or cytosine, as the starting materials by taking the following preliminary experiments into consideration: (a) 4-*aminosugars* could not be converted to the corresponding glycals through their halo derivatives, (b) an amino group could not be introduced into the C-4′ position of 2′,3′-unsaturated *uronic* nucleosides, and (c) silylated N-acetyl*cytosine* could be condensed with triacetylgalactal only in a very low yield and the acyl protecting group in cytosine is too unstable to carry out further modifications.

Condensation of triacetylgalactal (**4**) with 4 eq of silylated uracil **5** in the presence of 8 eq of $SbCl_5$ afforded an anomeric mixture of 1-(4′,6′-di-O-acetyl-2′,3′-dideoxy-D-*threo*-hex-2′-enopyranosyl)-uracils from which crystalline α-anomer **7** and amorphous β-anomer **6** could be isolated in yields of 40 and 24%, respectively, by means of column chromatography on silica gel. A trace amount of 3-uracilyl-3-deoxyglycal **8** was also obtained from the column; structure of the glycal being tentatively assigned to be 4,6-di-O-acetyl-1,2,3-trideoxy-3-(1′-uracilyl)-D-*xylo*-hex-1-enopyranose by analysis of NMR spectrum and from analogy of the previous work.[13] The β-anomer of the 2′-enopyranosyluracil **6** was deacetylated with sodium methoxide to the crystalline diol **9**. Assignment of the structure of the diol **9** was made unequivocally by derivation of it by selective mesylation followed by acetylation to the diol 4-acetate 6-mesylate **10**, whose NMR spectrum was completely superimposable with that of

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†To whom correspondence should be addressed.

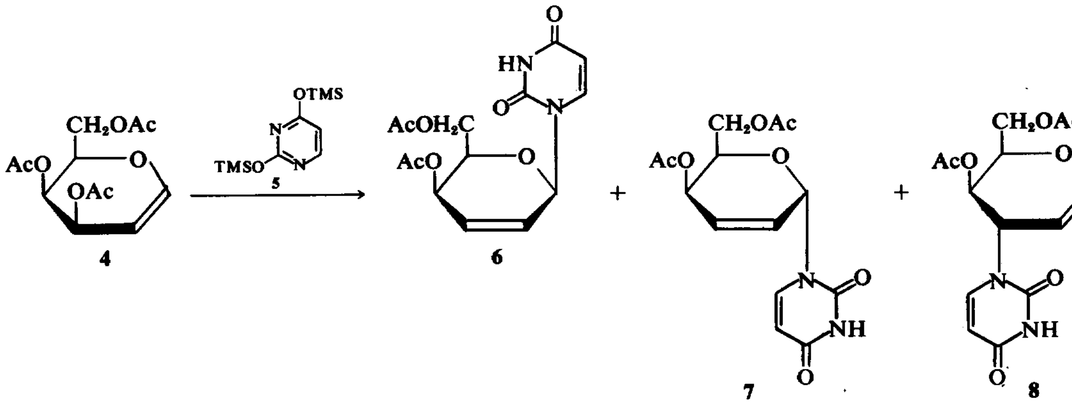
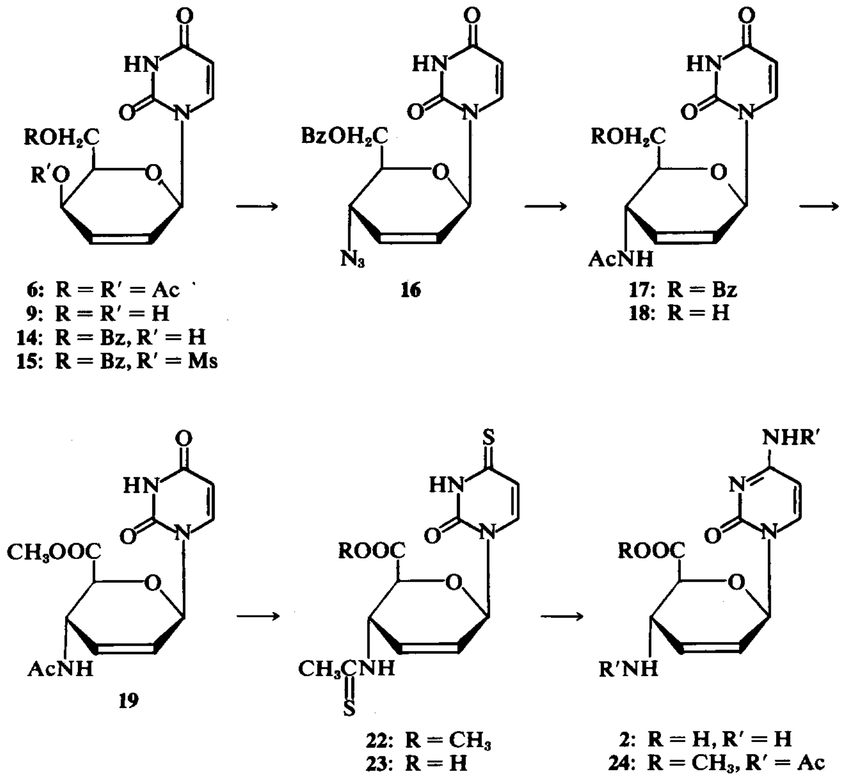


Fig 2.

the compound derived from the nucleoside 11[14] of known configuration by the following transformations: mesylation of 11 followed by selective displacement[15] with sodium benzoate gave 13, which was debenzoylated and acetylated.

Selective benzoylation of the diol 9 in pyridine with benzoyl chloride according to Reist *et al*[16] afforded the 6-monobenzoate 14 only in a low yield. The yield was improved to *ca* 50% by carrying out the reaction using 1% benzoyl chloride in CH_2Cl_2 at −20°. Mesylation of the 6-benzoate 14 with mesyl chloride and pyridine at −10° gave almost quantitatively the 4-mesylate 15. It is an allylic mesylate and strongly susceptible toward nucleophilic displacements and hence treatment of the mesylate with a saturated lithium azide solution in DMF at 0° for 15 min easily afforded the azide 16 in 78% yield. The signal of the anomeric proton in the azide 16 is split into quartet ($J = 2{\cdot}0$ Hz) by two olefinic protons and C-4′ proton, suggesting that the double bond is in its original 2′,3′-position. The coupling constant between 3′-H and 4′-H of the starting mesylate 15 is 5·0 Hz, whereas NMR of the azide 16 shows $J_{3',4'} = 2{\cdot}0$ Hz, thus strongly suggesting



6: R = R′ = Ac
9: R = R′ = H
14: R = Bz, R′ = H
15: R = Bz, R′ = Ms

17: R = Bz
18: R = H

22: R = CH_3
23: R = H

2: R = H, R′ = H
24: R = CH_3, R′ = Ac

Fig 3.

that the S_N2 reaction occurred at the C-4′ position and an *erythro* sugar was produced. When the reaction was carried out at a more elevated temp and/or for a longer reaction time this displacement reaction was accompanied partially by an allylic rearrangement[17] to give a 3′,4′-unsaturated 2′-azide.

Reduction of the azide group in **16** was accomplished with $CrCl_2$ according to Kirk and Wilson's method[18] to give the corresponding amine, which was isolated, after acetylation with acetic anhydride and pyridine, as the acetamide **17** in a 60% yield. Neutralization of the reduction mixture must be carried out carefully with $NaHCO_3$ aq to prevent O → N benzoyl migration. Sodium borohydride could also be used for reduction of the azide **16** but the yield was very low.[19] Debenzoylation of the amide **17** with methoxide in methanol gave the crystalline amidoalcohol **18** in quantitative yield.

Oxidation of the amidoalcohol **18** to the corresponding amidocarboxylic acid was achieved successfully by addition of a large excess of chromic anhydride to a solution of **18** in acetone-DMF at room temp and by allowing the resulting mixture to stand for 30 min. The reaction mixture was subsequently treated with excess diazomethane in ether and, after evaporation of the volatile solvents, the residue was chromatographed on a silica gel column to remove chromium compounds giving the amidocarboxylic acid methyl ester (N-acetyluracinine methyl ester;[1] **19**) in 42% yield. $J_{4',5'}$-value in the NMR spectrum of **19** is 9·0 Hz, thus confirming the *erythro* configuration at the C-4′ and C-5′ positions.

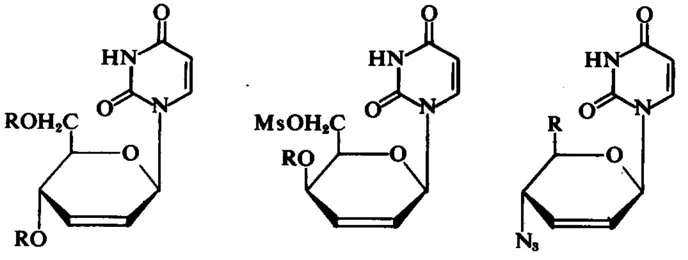
Conversion of the azide **16** into the uracinine derivative **19** by another route involving oxidation of the *prim* alcohol to an uronic acid followed by reduction of the azide group was also attempted. Thus, debenzoylation of **16** with sodium methoxide gave the azidoalcohol **20**, which was oxidized by the procedure described above to give the methyl azidouronate **21** in a yield of 81%; the structure **21** being confirmed from NMR [δ 3·91 ppm (3H, s)] and IR (2200 cm^{-1}) spectra. Reduction of **21** by $CrCl_2$, however, afforded an intractable mixture, from which the expected product **19** could not be isolated.

Conversion of the uracinine **19** into cytosinine (**2**) was accomplished by the procedure of Watanabe *et al.*[20] Thus, the ester **19** was refluxed in pyridine with P_2S_5 for 1 hr to give N-thioacetylthiouracinine methyl ester **22**, whose structure was suggested from the UV absorption bands at 268 and 328 nm. To prevent amide formation in the next amination step, the methyl ester **22** was hydrolyzed with 1 N NaOH in methanol at room temp to the carboxylic acid **23**, which was dissolved in methanol previously saturated with ammonia at −5° and heated in an autoclave at 100° for 24 hr. After evaporation of solvent, the residue was absorbed on an Amberlite IRA-410 (OH^- form) column. Elution of the column with 0·5 N HCl afforded cytosinine (**2**) as crystalline hydrochloride, IR spectrum of which (KBr disc) is superimposable to that of an authentic specimen prepared from blasticidin S (**1**) according to the method of Otake *et al.*[6] Further characterizations were made as follows: acetylation of cytosinine (**2**) with acetic anhydride and pyridine followed by methylation with diazomethane in ether afforded *N,N′*-diacetylcytosinine methyl ester (**24**). IR, UV, NMR and ORD spectra as well as m.p. of the synthetic **24** were identical with those of the authentic sample[6] prepared from blasticidin S,[1] thus confirming the structure including the absolute configuration.

EXPERIMENTAL

All m.ps are uncorrected. The following spectrometers were used: IR: JASCO IR-G and IR-E; UV: Hitachi EPS-3T; ORD: JASCO ORD/UV-5; NMR: JEOL 4H-100 and MH-100 (100 MHz). Chemical shifts (δ) are in ppm from int. TMS and coupling constants (J) in Hz (accuracy ±0·3 Hz). *J*-values marked by an asterisk were confirmed by double resonance experiments. TLC was performed on silica gel PF 254 (Merck) using CH_2Cl_2-MeOH (30:1) as the eluant, unless otherwise described. Silica gel (100 mesh, Kanto Chemical Co.) was used for column chromatography.

Condensation of triacetylgalactal (**4**) *and 2,4-bis(trimethylsilyl)uracil* (**5**). $SbCl_5$ (12 ml) was added to a stirred soln of **4** (4·58 g) and **5** (14·5 g) in dry EtOAc (200 ml) at room temp under an anhydrous condition. The temp of the mixture rose to about 50° and then the mixture was allowed to stand at room temp for 15 min. The soln was



11: R = H
12: R = Ms

13: R = Bz
10: R = Ac

20: R = CH₂OH
21: R = COOCH₃

Fig 4.

diluted with CH_2Cl_2 (450 ml), gradually poured into $NaHCO_3$ aq with stirring, and then the stirring was continued for further 1·5 hr. After the ppt was filtered off, the organic layer was separated, washed 4 times with $NaHCO_3$ aq, dried over Na_2SO_4 and evaporated to dryness. The residue was chromatographed on a silica gel column (250 g) by using CH_2Cl_2-MeOH (30 : 1) as the eluant to afford two anomers of the 2-enopyranosyl nucleosides and a trace amount of **8**. One of the anomers, 1-(4′,6′-di-O-acetyl-2′3′-dideoxy-β-D-*threo*-hex-2-enopyranosyl)uracil (**6**), was obtained as an amorphous solid (1·29 g; 24%); UV (MeOH) 258 nm (ε 9800); (MeOH–NaOH) 258 (7500); NMR ($CDCl_3$) 6·55 (H-1′), 5·92 (2′), 6·43 (3′), 5·17 (4′), 4·20 (5′ and 6′), 5·85 (5), 7·35 (6), 9·47 (NH), 2·16 (Ac), $J_{1',2'}$ = 1·5*, $J_{2',3'}$ = 10·0, $J_{3',4'}$ = 6·0*, $J_{4',5'}$ = ~ 0, $J_{1',3'}$ = 1·5*, $J_{1',4'}$ = 1·5*. The other anomer, 1-(4′,6′-di-O-acetyl-2′,3′-dideoxy-α-D-*threo*-hex-2-enopyranosyl)uracil (**7**), was crystallized from EtOH as needles (2·16 g; 40%), m.p. 148–149°. (Found: C, 51·46; H, 5·01; N, 8·51. $C_{14}H_{16}N_2O_7$ requires: C, 51·85; H, 4·97; N, 8·64%); UV (MeOH) 260 nm (ε 10500); (MeOH–NaOH) 260 (7900); NMR ($CDCl_3$) 6·45 (H-1′), 6·04 (2′), 6·55 (3′), 5·23 (4′), 4·4–3·9 (5′ and 6′), 5·71 (5), 7·32 (6), 9·61 (NH), 2·10 (Ac), 2·05 (Ac), $J_{1',2'}$ = 3·0*, $J_{2',3'}$ = 10, $J_{3',4'}$ = 5*, $J_{1',3'}$ = 2*, $J_{5,6}$ = 8. 4,6-Di-O-acetyl-1,2,3-trideoxy-3-(1′-uracilyl)-D-*xylo*(?)-hex-1-enopyranose (**8**): NMR ($CDCl_3$) 6·85 (H-1), 4·72 (2), 4·91 (4), 4·3–4·1 (5 and 6), 5·75 (5′), 7·52 (6′), 2·08 (Ac), 2·01 (Ac), $J_{1,2}$ = 6·5, $J_{2,3}$ = 5·0, $J_{1,3}$ = 1·5, $J_{5',6'}$ = 8.

1-(2′,3′-*Dideoxy-β-*D-threo-*hex-2′-enopyranosyl*)uracil (**9**). The diacetate **6** (2·56 g) was dissolved in MeOH containing 0·2 M NaOMe (75 ml) and the mixture was allowed to stand at room temp for 3 hr. The soln was neutralized with Amberlite IRC-50 (H^+ form), filtered and evaporated *in vacuo* to give the diol **9**, which was crystallized from isopropanol as prisms (1·29 g; 77%), m.p. 177–178°. (Found: C, 50·19; H, 5·04; N, 11·77. $C_{10}H_{12}N_2O_5$ requires: C, 50·00; H, 5·04; N, 11·66%); UV (MeOH) 259 nm (ε 11400).

1-(4′,6′-*Di-O-mesyl-2′,3′-dideoxy-β-*D-*erythro-hex-2′-enopyranosyl)uracil* (**12**). Mesyl chloride (2·0 ml) was added dropwise to a stirred soln of the diol **11**[12] (750 mg) in anhyd pyridine (20 ml). During addition the temp was kept below −5° and then maintained at 0° for 48 hr. Water (2 ml) was added to the soln at room temp to destroy excess mesyl chloride. After 2 hr the mixture was diluted with CH_2Cl_2 and water. The organic layer was washed successively with water and 1 N HCl, dried and evaporated to dryness. The residue was separated by TLC to give the dimesylate **12** as an amorphous solid (785 mg); NMR (CD_3COCD_3) 6·71 (H-1′), 6·25 (2′), 6·57 (3′), 5·17 (4′) 8·12 (NH), 4·6–4·3 (5′ and 6′), 5·70 (5), 7·40 (6), 3·25 (CH_3SO_2), 3·12(CH_3-SO_2).

1-(4′-O-*Benzoyl-6′-mesyl-2′,3′-dideoxy-β-*D-threo-*hex-2′-enopyranosyl)uracil* (**13**). The dimesylate **12** (53 mg) and sodium benzoate (26 mg) (freshly fused before use) was dissolved under stirring in hexamethylphosphortriamide (1·5 ml). After being kept at room temp for 12 hr, the mixture was diluted with CH_2Cl_2, thoroughly washed with water and evaporated. Separation by TLC afforded the monobenzoate **12** as an amorphous solid (30 mg); NMR ($CDCl_3$) 6·26 (H-1′), 5·99 (2′), 6·60 (3′), 5·44 (4′), 4·42 (5′ and 6′), 7·30 (6), 5·77 (5), 8·05 and 7·5 (5H, arom), 3·03 (CH_3SO_2), $J_{1',2'}$ = ~ 0, $J_{2',3'}$ = 10, $J_{1'3'}$ = 1, $J_{3',4'}$ = 5, $J_{4',5'}$ = ~ 0, $J_{5,6}$ = 8.

1-(4′-O-*Acetyl-6′-O-mesyl-2′,3′-dideoxy-β-*D-threo-*hex-2′-enopyranosyl)uracil* (**10**). (a) *From the monobenzoate* **13**. Compound **13** (16 mg) was dissolved in MeOH containing 0·01 M NaOMe (1·5 ml). After 1 hr, the mixture was neutralized with Amberlite IRC-50 (H^+ form) and evaporated to dryness. The residue was acetylated with Ac_2O and pyridine to afford **10**; NMR ($CDCl_3$) 6·54 (H-1′), 5·95 (2′), 6·46 (3′), 5·21 (4′), 4·31 (5′ and 6′). 5·80 (5), 7·27 (6), 9·1 (NH), 3·05 (CH_3SO_2), 2·13 (Ac). (b) *From the diol* **9**. Mesyl chloride (10 μl) was added in one portion to a stirred soln of **9** (15 mg) in pyridine (0·4 ml) at −20° and then the soln was kept at 0° until the starting material was almost disappeared (monitored by TLC). Evaporation followed by acetylation with Ac_2O and pyridine afforded the crude **10**, which was purified by TLC. NMR spectrum of **10** obtained from **9** is completely superimposable to that of **10** obtained from **13**.

1-(6′-O-*Benzoyl-2′,3′-dideoxy-β-*D-threo-*hex-2′-enopyranosyl)uracil* (**14**). To a well stirred soln of **9** (1·57 g) in anhyd pyridine (60 ml) was added dropwise a soln of benzoyl chloride in CH_2Cl_2 (1%, 250 ml) at −40° and then temp of the mixture was kept at −20°. When the starting material had almost disappeared (monitored by TLC), the mixture was treated with dry MeOH (50 ml) at −10° and then evaporated *in vacuo* below 5°. The residue was chromatographed on a silica gel column by use of CH_2Cl_2–MeOH (30 : 1) as the eluant, and then crystallized from isopropanol to give **14** (1·05 g; 49%), m.p. 175°. (Found: C, 59·69; H, 4·64; N, 8·25. $C_{17}H_{16}N_2O_6$ requires: C, 59·30; H, 4·68; N, 8·14%); NMR ($CDCl_3$) 6·49 (H-1′), 5·85 (2′), 6·49 (3′), 4·15 (4′ and 5′), 4·70 and 4·50 (6′), 5·80 (5), 8·1 and 7·5 (arom), $J_{2',3'}$ = 10, $J_{5,6}$ = 8.

1-(6′-O-*Benzoyl-4′-O-mesyl-2′,3′-dideoxy-β-*D-threo-*hex-2′-enopyranosyl)uracil* (**15**). To a soln of **14** (808 mg) in a mixture of anhyd pyridine (12 ml) and CH_2Cl_2 (20 ml) was added dropwise at −40° mesyl chloride (6 ml). The temp of the mixture was allowed to rise to −10° and then kept at this temp until the reaction was completed (monitored by TLC). The soln was diluted with CH_2Cl_2 (100 ml), washed successively with ice-cold water, cold 1 N HCl and $NaHCO_3$ aq, and evaporated below 40°. For removal of excess mesyl chloride, the residue was chromatographed on a short silica gel column using CH_2Cl_2–MeOH (30 : 1) as the eluant. The eluted soln was evaporated at a temp as low as possible to give **15** as an amorphous solid (925 mg, *ca* 100%), NMR ($CDCl_3$) 6·66 (H-1′), 6·07 (2′), 6·56(3′), 5·13 (4′), 4·6–4·3 (5′ and 6′), 5·78 (5), 7·35 (6), 9·33 (NH), 7·95 and 7·45 (arom), 3·12 (CH_3SO_2), $J_{2',3'}$ = 10, $J_{3',4'}$ = 5*, $J_{1',3'}$ = 1*, $J_{5,6}$ = 8.

1-(6′-O-*Benzoyl-4′-azido-2′,3′,4′-trideoxy-β-*D-erythro-*hex-2′-enopyranosyl)uracil* (**16**). The mesylate **15** (1·61 g) was added at −5° in a soln of anhyd LiN_3 (12 g) in dry DMF (120 ml), and the mixture was allowed to stand at 0° for 15 min. The soln was poured onto stirred ice-cold water and extracted with CH_2Cl_2. The extract was repeatedly washed with ice-cold water to remove DMF, dried over Na_2SO_4 and evaporated *in vacuo* below 15°. The residue was purified by preparative TLC to give the azide **16** as an amorphous solid (1·10 g, 78%); NMR ($CDCl_3$) 6·58 (H-1′), 5·89 (2′), 6·35 (3′), 4·3–3·8 (4′ and 5′), 4·60 and 4·58 (6′), 5·75 (5), 7·25 (6), 8·14 and 7·58 (arom), $J_{1',2'}$ = 2·0, $J_{2',3'}$ = 10, $J_{3',4'}$ = 2·0*, $J_{1',3'}$ = 2·0*, $J_{1',4'}$ = 2·0*, $J_{5',6'}$ = 2·5 and 5·0, $J_{6'(gem)}$ = 14·0, $J_{5,6}$ = 8; IR ($CHCl_3$) 2200 cm^{-1}.

1-(4′-*Acetamido-6′-O-benzoyl-2′,3′,4′-trideoxy-β-*D-erythro-*hex-2′-enopyranosyl)uracil* (**17**). A chromous chloride soln (158 ml) prepared according to the method of Rosenkranz *et al*[21] was gradually added to a stirred soln of **16** (2·43 g) in a mixture of acetone and water (2 : 1, 110 ml) under N_2 at at room temp. During the addition N_2 gas evolved from the soln. After 10 min, the mixture was carefully neutralized with $NaHCO_3$ aq, filtered and evaporated to dryness *in vacuo*. Repeated extractions from the residue with CH_2Cl_2–MeOH (1:1) gave an extract which, after being passed through a very short column of silica gel, was evaporated to dryness to afford a crude amine, which had no azide absorption band in its IR.

The amine was treated with Ac_2O and pyridine (1:2, 450 ml) overnight, and the soln was evaporated to dryness. The residue was purified by column chromatography using silica gel to give the acetamide **17** as an amorphous solid (1·50 g, 60%); NMR ($CDCl_3$) 6·58 (H-1′), 5·70 (2′), 6·18 (3′), 5·0–4·0 (4′, 5′ and 6′), 5·73 (5), 7·22 (6), 9·9 (NH), 6·59 (NH), 8·03 and 7·6 ~ 7·3 (arom), $J_{2',3'}$ = 10, $J_{5,6}$ = 8.

1-(4′-*Acetamido*-2′,3′,4′-*trideoxy*-β-D-erythro-*hex*-2′-*enopyranosyl)uracil* (**18**). The amide **17** was dissolved at room temp in a MeOH soln of 0·1 M NaOMe (30 ml). After 12 hr the soln was neutralized with Amberlite IRC-50 (H^+ form) and evaporated to dryness to give crude amido-alcohol **18** (1·3 g), which was crystallized from water as needles, m.p. 202°. (Found: C, 51·46; H, 5·60; N, 15·16. $C_{12}H_{15}N_3O_5$ requires: C, 51·24; H, 5·38; N, 14·94%).

N-*Acetyluracinine methyl ester* (**19**). To a stirred soln of **18** (1·0 g) in acetone-DMF (1:1, 200 ml) was added chromic anhydride (65 g) in small portions. During the addition the temp was prevented from vigorous rising by cooling with water and then the mixture was allowed to stand at room temp for 30 min. It was then diluted with MeOH and treated with an ether soln of a large excess of diazomethane for 10 min. After evaporation of the volatile solvents at room temp under reduced pressure, the residual soln was applied to a column of silica gel (500 g) to remove chromium compounds, and the products were eluted with CH_2Cl_2–MeOH (93:7). The eluents were evaporated to dryness and purified by preparative TLC to give **19** as an amorphous solid (460 mg, 42%); NMR ($CDCl_3$–CD_3OD 1:1) 6·65 (H-1′), 5·95 (2′), 6·28 (3′), 4·38 (5′), 5·90 (5), 7·58 (6), 3·80 (CH_3O), 2·00 (CH_3CO), $J_{2',3'}$ = 10, $J_{4',5'}$ = 9, $J_{5,6}$ = 8.

1-(4′-*Azido*-2′,3′,4′-*trideoxy*-β-D-*hex*-2′-*enopyranosyl*)-*uracil* (**20**). The monobenzoate **16** (48 mg) was dissolved in 0·04 M NaOMe in MeOH (5 ml) at room temp. After 2 hr, the soln was neutralized with Amberlite IRC-50 (H^+ form) and evaporated to dryness. TLC separations afforded **20** (22 mg) as an amorphous solid, NMR ($CDCl_3$) 6·58 (H-1′), 5·89 (2′), 6·38 (3′), 4·3–3·8 (4′ and 5′), 4·60 and 4·58 (6′), 8·10 and 7·58 (arom), 5·75 (5), 7·25 (6), $J_{1',2'}$ = 1·5, $J_{2',3'}$ = 10, $J_{3',4'}$ = 1·5, $J_{1',3'}$ = 1·5, $J_{2',4'}$ = 1·5, $J_{5,6}$ = 8; IR ($CHCl_3$) 2200 cm^{-1}.

Methyl 1-(1′-*uracilyl*)-4-*azido*-2,3,4-*trideoxy*-β-D-erythro-*hex*-2-*enopyranuronate* (**21**). Chromic anhydride (600 mg) was added in small portions to a soln of **20** (10 mg) in acetone–DMF (1:1, 12 ml) at room temp during 30 min. The mixture was diluted with MeOH, treated with excess diazomethane in ether, evaporated and extracted with CH_2Cl_2. TLC separations afforded the ester **21** as an amorphous solid (9 mg, 81%); NMR ($CDCl_3$) 6·57 (H-1), 5·87 (2), 6·23 (3), 4·46 (4), 4·39 (5), 5·77 (5′), 7·12 (6′), 3·91 (CH_3O); IR (KBr) 2200 cm^{-1}.

N-*Thioacetylthiouracinine methyl ester* (**22**). P_2S_5 (1·17 g) was added to a soln of **19** (447 mg) in anhyd pyridine (120 ml), and the mixture was gently refluxed for 1 hr. It was then evaporated to dryness and the residue was dissolved in CH_2Cl_2. After most of the excess P_2S_5 was removed by passing through a short silica gel column, the CH_2Cl_2 soln was evaporated to dryness and the residue was subjected to TLC separations to give **22** (226 mg, 46%) and N-acetylthiouracinine methyl ester (30 mg); UV (MeOH) 275 nm. The former compound (**22**) was crystallized from EtOH as yellow needles, m.p. 204–205°. (Found: C, 45·86; H, 4·68; N, 12·53. $C_{13}H_{15}N_3O_4S_2$ requires: C, 45·75; H, 4·43; N, 12·31%); UV (MeOH) 268 nm (ε 17000), 328 (21200); NMR ($CDCl_3$–CD_3OD 1:1) 6·50 (H-1′), 5·82 (2′), 6·25 (3′), 5·68 (4′), 4·55 (5′), 6·40 (5), 7·23 (6), 3·71 (CH_3O), 2·48 (CH_3CS), $J_{1',2'}$ = 1·5, $J_{2',3'}$ = 10, $J_{3',4'}$ = 1·5*, $J_{4',5'}$ = 9*, $J_{1',3'}$ = 1·5, $J_{1',4'}$ = 1·5*, $J_{2',4'}$ = 1·5*, $J_{5,6}$ = 8.

N-*Thioacetylthiouracinine* (**23**). To a soln of **22** (100 mg) in MeOH (54 ml) was added 1 N NaOH (25 ml), and the mixture was allowed to stand at room temp until the reaction was completed (monitored by TLC). It was then neutralized with Amberlite IRC-50 (H^+ form) and extracted repeatedly with MeOH–H_2O (1:1). The extract was evaporated to dryness to give **23** (60 mg, 63%) as an amorphous solid; NMR (CD_3OD) 6·06 (H-1′), 5·98 (2′), 6·45 (3′), 5·68 (4′), 4·38 (5′), 6·55 (5), 7·55 (6), 2·55 (CH_3CS), $J_{2',3'}$ = 10, $J_{4',5'}$ = 9*, $J_{5,6}$ = 8.

Cytosinine dihydrochloride (**2**). The uracinine **23** (50 mg) and a saturated soln of ammonia in anhyd MeOH (12 ml) at −5° were charged under anhyd conditions in a stainless steel autoclave and heated at 100° for 40 hr. After removal of the solvent, the residue was absorbed on Amberlite IRA-410 (OH^- form) and subsequently eluted with 0·5 N HCl. The fractions which showed an UV absorption at 260 nm were combined, carefully evaporated to a small volume, and stored at 0°, when crystalline **2** precipitated; NMR (D_2O; DSS as standard) 6·64 (H-1′), 6·25 (2′), 6·50 (3′), 6·25 (5), 7·76 (6). This compound was identified by comparison of its IR spectrum with the authentic sample derived from blasticidin S.[6]

Diacetylcytosinine methyl ester (**24**). (a) *From* **23**. Compound **23** (10 mg) was treated with methanolic ammonia as described. After evaporation of the solvents, the residue was directly acetylated with Ac_2O and pyridine (1:2; 1·5 ml) at room temp overnight. After evaporation of the solvent the product was dissolved in MeOH and esterified with an ether soln of excess diazomethane. The mixture was evaporated *in vacuo* and separated by TLC to give **24** (2·6 mg), which was crystallized from water as needles, m.p. 267–268° (dec); UV (MeOH) 251 nm (ε 14200), 300 (5820); (MeOH-1 N HCl) 247 (8740), 309 (9300); ORD [Φ] (peak at 257 nm) + 3200 (dioxane-EtOH 1:1); NMR ($CDCl_3$–CD_3OD 1:1) 6·75 (H-1′), 6·00 (2′), 6·25 (3′), 5·00 (4′), 4·45 (5′), 7·63 (5), 8·02 (6), 3·80 (CH_3O), 2·28 (CH_3CO), 2·00 (CH_3CO), $J_{1',2'}$ = 1·5, $J_{2',3'}$ = 10, $J_{3',4'}$ = 1·5, $J_{4',5'}$ = 9, $J_{1',3'}$ = 1·5, $J_{1',4'}$ = 1·5, $J_{5,6}$ = 8.

(b) *From blasticidin S*. Cytosinine dihydrochloride derived from blasticidin S was converted to **24** in the same method as described above, m.p. 263–267° (dec); ORD [Φ] (peak at 257 nm) + 3400 (dioxane–EtOH 1:1); UV (MeOH) 251 nm (ε 14600), 300 (6060); (MeOH-1 N HCl) 247 (9020), 309 (9580).

IR and NMR spectra of both diacetylcytosinine methyl esters obtained from synthetic cytosinine and from blasticidin S were superimposable each other.

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