Aminoacyl and Dihydro-derivatives of Isocytidine and Isocytosine

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Isocytosine and 2',3'-*O*-protected isocytidines were coupled with appropriately protected L-phenylalanine, glycine, or glycylglycine yielding the corresponding 2-*N*-aminoacyl-isocytosines and -isocytidines. The mode of hydrolysis of 2-*N*-(*N*-t-butoxycarbonyl-L-phenylalanyl)isocytidine (5) with trifluoroacetic acid, to give 2-*N*-L-phenylalanylisocytidine (16), 2-*N*-L-phenylalanylisocytosine (17), and minor amounts of unexpected 5'-*O*-phenylalanyl derivatives, (18) and (19), was shown to depend on the reaction conditions.

Catalytic hydrogenation of 2',3'-*O*-ethoxymethyleneisocytidine (9), the 2-*N*-aminoacylisocytidines (5) and (7), and 2-*N*-glycylglycylisocytosine (15) over 5% rhodium–carbon gave the corresponding 5,6-dihydro-derivatives (20), (23), (22), and (21) in quantitative yields.

ISOCYTIDINE [1] and isocytosine [2] are of both biophysical and biochemical interest. The unusual crystals of isocytosine, containing two tautomers in an exact 1 : 1 ratio,[3] show ready proton migration in the presence of water vapour, and transmit currents enormously greater than those usually associated with organic semiconductors.[4] U.v. spectroscopic data [5] and the *X*-ray crystal structure of 5,6-dihydroisocytidine monohydrate have revealed the molecule to exist as the amino-tautomer state.[6] The utilization of isocytidine by living cells as a pyrimidine source,[7] the great quantities of aminoacyl and peptidyl components firmly bound to deoxyribonucleic acids in tumours,[8] and the possible 'punctuation' role of such components in the genetic

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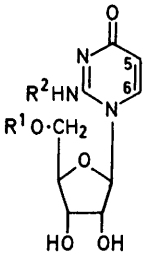
code [9] (acting as ' derepressors ' of structural genes [10]) directed our studies towards the synthesis of 2-*N*-aminoacyl-isocytidine and -isocytosine derivatives.

Acetylation of 2′,3′-*O*-isopropylidene isocytidine [1,11] with 1 equiv. of acetic anhydride yielded the 2-*N*-acetyl derivative (2) (73.2%) and only 30.7% of the 2-*N*,5′-*O*-diacetyl derivative (3). The successful separation of the 2-*N*-acetylisocytidine (2) encouraged us to attempt the preparation and separation of 2-*N*-aminoacyl derivatives. Thus, 2-*N*-(*N*-benzyloxycarbonyl-DL-phenylalanyl)- (4), 2-*N*-(*N*-t-butoxycarbonyl-L-phenylalanyl)- (5), and 2-*N*-(*N*-t-butoxycarbonylglycyl)- (7) 2′,3′-*O*-isopropylideneisocytidines were



(12) R^1 = H, R^2 = Boc-L-Phe

2′,3′-*O*-CHOEt derivatives

(9) R^1 = R^2 = H

(10) R^1 = H, R^2 = Boc-L-Phe

(20) R^1 = R^2 = H; 5,6-dihydro

2′,3′-*O*-CMe_2 derivatives

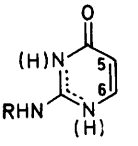
(1) R^1 = R^2 = H
(2) R^1 = H, R^2 = Ac
(3) R^1 = R^2 = Ac
(4) R^1 = H, R^2 = Z-DL-Phe
(5) R^1 = H, R^2 = Boc-L-Phe
(6) R^1 = R^2 = Boc-L-Phe
(7) R^1 = H, R^2 = Boc-Gly
(8) R^1 = R^2 = Boc-Gly
(11) R^1 = Ac, R^2 = Boc-L-Phe
(13) R^1 = H, R^2 = $(Gly)_2CPh_3$
(16) R^1 = H, R^2 = L-Phe,CF_3CO_2H
(18) R^1 = Boc-L-Phe, R^2 = H
(19) R^1 = L-Phe,CF_3COOH, R^2 = H
(22) R^1 = H, R^2 = Boc-Gly; 5,6-dihydro
(23) R^1 = H, R^2 = Boc-HNCHCO; 5,6-dihydro
$C_6H_{11}CH_2$

Z = $PhCH_2$·O·CO
Boc = Bu^tO·CO

easily separated (63—67%) from the reaction of the isocytidine (1) with appropriately protected DL-phenylalanine, L-phenylalanine, or glycine in the presence of dicyclohexylcarbodi-imide.[12] The 2-*N*,5′-*O*-bis-L-phenylalanyl (6) and diglycyl (8) derivatives were isolated in 17—20% yield. The de-*O*-acetylation of these by-products is exemplified by the hydrolysis with concentrated ammonia–dioxan (1 : 1) of the 2-*N*,5′-*O*-bis-(aminoacyl)isocytidine (6) to give the 2-*N*-aminoacyl derivative (5). Acetylation of compound (5) yielded 5′-*O*-acetyl-2′,3′-*O*-isopropylidene-2-*N*-(*N*-t-butoxycarbonyl-L-phenylalanyl)isocytidine (11).

Aminoacylation of the hitherto unknown acid-labile 2′,3′-*O*-ethoxymethyleneisocytidine (9) conveniently afforded the corresponding 2-*N*-(*N*-t-butoxycarbonyl-L-phenylalanyl) derivative (10) in 71% yield. The n.m.r. spectra of the 2′,3′-*O*-ethoxymethylene ribonucleosides (9) and (10) agree with those reported for the diastereoisomeric 2′,3′-*O*-benzylidene nucleosides.[13]

In contrast to earlier investigations of cytidine,[14] the coupling reactions of unprotected isocytidine, with *NN*′-dicyclohexylcarbodi-imide as condensing agent, afforded numerous side products and only a 17% yield of 2-*N*-(*N*-t-butoxycarbonyl-L-phenylalanyl)isocytidine (12). 2′,3′-*O*-Isopropylidene-2-*N*-(*N*-triphenylmethylglycylglycyl)isocytidine (13) was also obtained, in poor yield, when the hydroxysuccinimido-ester of *N*-triphenylmethylglycylglycine [15-17] was coupled with 2′,3′-*O*-isopropylideneisocytidine. However, isocytosine was converted into 2-*N*-(*N*-triphenylmethylglycylglycyl)isocytosine (14) in 80% yield. Detritylation of compound (14) with 50% acetic acid gave 2-*N*-glycylglycylisocytosine (15).



(14) R = $(Gly)_2CPh_3$
(15) R = H-$(Gly)_2$
(17) R = L-Phe
(21) R = $(Gly)_2$; 5,6-dihydro

Treatment of compound (5) with 98% trifluoroacetic acid at −12 °C for 5 min afforded crystalline 2′,3′-*O*-isopropylidene-2-*N*-L-phenylalanylisocytidine trifluoroacetate salt (16). *N* ⟶ *O*-Acyl migration brought about by strong acids has been widely investigated.[18-20] In our experience hydrolysis of compound (5) with 98% trifluoroacetic acid at 23 °C for 5 min led to cleavage of the *N*-glycosyl bond and isolation of 2-*N*-L-phenylalanylisocytosine (17). The formation of minor amounts of 5′-*O*-(*N*-t-butoxycarbonyl-L-phenylalanyl)-2′,3′-*O*-isopropylideneisocytidine (18) and 5′-*O*-L-phenylalanyl-2′,3′-*O*-isopropylideneisocytidinetrifluoroacetate salt (19) can be explained in terms of 2-*N* ⟶ 5′-*O*-aminoacyl migration. The marked influence of the 5′-*O*-acyl group on the chemical shift of the C-6 proton was of diagnostic value in the identification of aminoacylated products.

In continuation of our studies on the chemistry of

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aminopyrimidine nucleosides [5,21] we have found that hydrogenation of 2′,3′-*O*-ethoxymethyleneisocytidine (9), 2-*N*-glycylglycylisocytosine (15), and the 2-*N*-glycylisocytidine (8) over 5% rhodium–carbon [22] affords the 5,6-dihydro-derivatives in quantitative yields. Hydrogenation of the 2-*N*-phenylalanylisocytidine (5) gave 2′,3′-*O*-isopropylidene-2-*N*-(*N*-t-butoxycarbonyl-L-β-cyclohexylalanyl)-5,6-dihydroisocytidine (23).

EXPERIMENTAL

The same techniques and apparatus were used as previously described.[23] In addition optical rotations were measured for solutions in anhydrous ethanol (*l* 1 dm) with a Zeiss–Winkel 179707 apparatus.

Acetylation of 2′,3′-O-*Isopropylideneisocytidine* [1] (1).—A solution of isocytidine (1) (142 mg, 0.5 mmol) in anhydrous pyridine (3 ml) and acetic anhydride (0.05 ml, 0.5 mmol) was kept at room temperature for 48 h and then evaporated to dryness. Preparative t.l.c. [three developments in methylene chloride–methanol (20 : 1); eluant acetone] separated starting material (40 mg; R_F *ca.* 0.1); a foam identified as 2-*N*-acetyl-2′,3′-*O*-isopropylideneisocytidine (2) (86 mg, 73.2% based on transformed isocytidine), m.p. 146—147 °C (from dichloromethane–ether) (lit.,[24] 145—148 °C); R_F *ca.* 0.5; $[\alpha]_D^{20}$ −47.5° (*c* 0.54); dried at 80 °C and 10^{-5} mmHg (Found: C, 51.4; H, 6.1; N, 12.7. Calc. for $C_{14}H_{19}N_3O_6$: C, 51.7; H, 5.9; N, 12.9%); $\lambda_{max.}$ 209, 255, and 275sh nm (log ε 4.07, 4.19, and 4.04), $\lambda_{min.}$ 232 nm (log ε 3.81); $\nu_{max.}$ 3 571br, 3 040, 1 698, 1 642, and 1 587br cm^{-1}; τ(CD_3OD) 1.91 (1 H, d, 6-H; $J_{6.5}$ 8.2 Hz), 4.10 (1 H, d, 5-H; $J_{5.6}$ 8.2 Hz), 7.86 (3 H, s, NAc), 8.45 and 8.65 (2 × 3 H, s, CMe_2); and the foamy 2-*N*,5′-O-*diacetyl derivative* (3) (30 mg, 30.7%), R_F *ca.* 0.7, which was also obtained (169 mg, 92%) from isocytidine (1) (142 mg, 0.5 mmol) with 2 equiv. of acetic anhydride (0.1 ml, 1.0 mmol); $[\alpha]_D^{24}$ −4.9° (*c* 0.8) (Found: C, 52.3; H, 6.05; N, 11.7. $C_{16}H_{21}N_3O_7$ requires C, 52.3; H, 5.75; N, 11.45%); $\lambda_{max.}$ 212, 254, and 273sh nm (log ε 4.04, 4.25, and 4.13), $\lambda_{min.}$ 232 nm (log ε 3.81); $\nu_{max.}$ 3 096, 3 003, 1 730, 1 689, 1 631, and 1 575 cm^{-1}; τ(CD_3CD) 2.17 (1 H, d, 6-H; $J_{6.5}$ 8.2 Hz), 4.07 (1 H, d, 5-H; $J_{5.6}$ 8.2 Hz), 7.83 (3 H, s, NAc), 7.97 (3 H, s, OAc), and 8.44 and 8.66 (2 × 3 H, s, CMe_2).

2-N-*Aminoacylation of Isocytidines.—General procedure.* Isocytidine, its 2′,3′-*O*-isopropylidene derivative,[1] or its 2′,3′-*O*-ethoxymethylene derivative (1 mmol) in pyridine (2 ml) was treated at room temperature for 48 h with the *N*-benzyloxycarbonyl or *N*-t-butoxycarbonyl derivative of phenylalanine or glycine (1.2 mmol) in the presence of a solution in dioxan (5 ml) of *NN*′-dicyclohexylcarbodi-imide (1.3 mmol). The precipitate was filtered off and washed with methanol, and the filtrate evaporated to dryness. Preparative t.l.c. (silica gel) [four developments in methylene chloride–methanol (10 : 1); eluant methanol] separated starting material (*ca.* 20%) and foamy products (R_F *ca.* 0.4—0.5).

2-N-(*N-Benzyloxycarbonyl-*DL-*phenylalanyl*)-2′,3′-O-*Isopropylideneisocytidine* (4) (65%) showed $[\alpha]_D^{22}$ −8.9° (*c* 0.9 in CH_2Cl_2) (Found: C, 61.5; H, 5.8; N, 9.7. $C_{29}H_{32}N_4O_8$ requires C, 61.7; H, 5.7; N, 9.9%); $\lambda_{max.}$ 206, 255, and 287.5 nm (log ε 4.41, 4.22, and 4.18), $\lambda_{min.}$ 234 and 265 nm (log ε 3.99 and 4.13); $\nu_{max.}$ 3 460, 3 344, 2 933, 1 692br, 1 626, 1 570br, and 698 cm^{-1}.

2′,3′-O-*Isopropylidene*-2-N-(*N-t-butoxycarbonyl-*L-*phenylalanyl*)*isocytidine* (5). (*a*) This *product* (67%) had m.p. 150—152 °C (from acetone–n-hexane); R_F *ca.* 0.5; $[\alpha]_D^{18}$ −38.2° (*c* 1) (Found: C, 59.05, H, 6.75; N, 10.4. $C_{26}H_{34}N_4O_8$ requires C, 58.85; H, 6.45; N, 10.55%); $\lambda_{max.}$ 204, 254.5, and 276 nm (log ε 4.30, 4.26, and 4.20), $\lambda_{min.}$ 232 and 265 nm (log ε 3.94 and 4.17); $\nu_{max.}$ 3 521, 3 436, 3 145, 3 021, 1 715, 1 681, 1 631br, 1 580br, and 701 cm^{-1}; τ 1.99 (1 H, d, 6-H; $J_{6.5}$ 8.3 Hz), 2.80 (5 H, s, aromatic), 4.12 (1 H, d, 5-H; $J_{5.6}$ 8.3 Hz), 8.37 and 8.63 (2 × 3 H, s, CMe_2), and 8.64 (9 H, s, Bu^t). 2′,3′-O-*Isopropylidene*-2-N,5′-O-*bis*-(*N-t-butoxycarbonyl-*L-*phenylalanyl*)*isocytidine* (6) (17%), also obtained from this aminoacylation, showed R_F *ca.* 0.7; $[\alpha]_D^{18}$ −13.9° (*c* 1) (Found: C, 61.95; H, 6.85; N, 9.05. $C_{40}H_{51}N_5O_{11}$ requires C, 61.75; H, 6.6; N, 9.0%); $\lambda_{max.}$ 206.5, 255.5, and 274 nm (log ε 4.67, 4.54, and 4.50), $\lambda_{min.}$ 234 and 266 nm. (log ε 4.14 and 4.46); $\nu_{max.}$ 3 401, 2 985, 1 754sh, 1 704, 1 639, 1 580, and 699 cm^{-1}; τ 2.38 (1 H, d, 6-H; $J_{6.5}$ 8.3 Hz), 2.75—2.81 (5 H, m, aromatic), 4.08 (1 H, d, 5-H; $J_{5.6}$ 8.3 Hz), 8.41 and 8.67 (2 × 3 H, s, CMe_2), and *ca.* 8.60br (2 × 9 H, Bu^t).

(*b*) Treatment of the bisaminoacylisocytidine (6) with concentrated ammonia–dioxan (1 : 1) at room temperature for 4 h gave a crystalline product (73%), m.p. 150—152 °C, identical (mixed m.p. and i.r. spectrum) with the product from (*a*).

2′,3′-O-*Isopropylidene*-2-N-(*N-t-butoxycarbonylglycyl*)*isocytidine* (7). This *product* (63%) exhibited $[\alpha]_D^{22}$ −22.6° (*c* 0.7) (Found: C, 51.95; H, 6.65; N, 12.7. $C_{19}H_{28}N_4O_8$ requires C, 51.8; H, 6.4; N, 12.7%); $\lambda_{max.}$ 214, 256, and 273sh nm (log ε 3.94, 4.17, and 4.07), $\lambda_{min.}$ 235 nm (log ε 3.73); $\nu_{max.}$ 3 401br, 3 125, 2 985, 1 698br, 1 634, and 1 577 cm^{-1}; τ 2.04 (1 H, d, 6-H; $J_{6.5}$ 8.3 Hz), 4.18 (1 H, d, 5-H; $J_{5.6}$ 8.3 Hz), 8.38 and 8.61 (2 × 3 H, s, CMe_2), and 8.58 (9 H, s, Bu^t). 2′,3′-O-*Isopropylidene*-2-N,5′-O-*bis*-(*N-t-butoxycarbonylglycyl*)*isocytidine* (8), also isolated from this aminoacylation (20%), had R_F *ca.* 0.6; $[\alpha]_D^{22}$ −12.5° (*c* 0.7) (Found: C, 52.1; H, 6.8; N, 11.45. $C_{26}H_{39}N_5O_{11}$ requires C, 52.25; H, 6.6; N, 11.7%); $\lambda_{max.}$ 210, 253, and 271sh nm (log ε 4.37, 4.56, and 4.46), $\lambda_{min.}$ 231 nm (log ε 4.19); $\nu_{max.}$ 3 401, 2 976, 1 748sh, 1 695br, 1 634, and 1 577br cm^{-1}; τ 2.38 (1 H, d, 6-H; $J_{6.5}$ 8.3 Hz), 4.05 (1 H, d, 5-H; $J_{5.6}$ 8.3 Hz), 8.37 and 8.62 (2 × 3 H, s, CMe_2), and 8.56 and 8.59 (2 × 9 H, s, 2 × Bu^t).

2′,3′-O-*Ethoxymethyleneisocytidine* (9).—A solution of isocytidine (729 mg, 3 mmol) in anhydrous dioxan (20 ml) was treated with trichloroacetic acid (1.3 g) and triethyl orthoformate [25] (1.5 ml) at 50 °C for 3 h, then cooled, neutralized [ion-exchange resin IR-4B (OH^-) (100 ml) in dioxan], and evaporated to dryness. The residue was chromatographed on a silica gel (40 g) column in methylene chloride. Methylene chloride–methanol (10 : 1) eluted a *foam* (775 mg, 86%), R_F *ca.* 0.2 [t.l.c. in methylene chloride–methanol (10 : 1)]; $[\alpha]_D^{22}$ −76.4° (*c* 1.36); dried at 60 °C and 10^{-5} mmHg (Found: C, 47.9; H, 5.95; N, 13.75. $C_{12}H_{17}N_3O_6$ requires C, 48.15; H, 5.75; N, 14.05%); $\lambda_{max.}$ 209, 235sh, and 258sh nm (log ε 4.54, 4.15, and 3.87); $\nu_{max.}$ 3 448br, 3 289, 3 030, 1 656, and 1 629 cm^{-1}; τ(CD_3OD) 2.31

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and 2.33 (1 H, d, 6-H; $J_{6.5}$ 8.0 Hz), 4.29 and 4.33 (1 H, d, 5-H; $J_{5.6}$ 8.0), 6.30 and 6.38 (2 H, q, CH_2O; J_{Et} 7 Hz), 8.77 and 8.88 (3 H, t, CH_3; J_{Et} 7 Hz).

2′,3′-O-*Ethoxymethylene*-2-N-(N-*t-butoxycarbonyl*-L-*phenylalanyl*)*isocytidine* (10).—To a solution of 2′,3′-*O*-ethoxymethyleneisocytidine (9) (450 mg, 1.5 mmol) in anhydrous dioxan (15 ml), *N*-t-butoxycarbonyl-L-phenylalanine (480 mg, 1.8 mmol) and dicyclohexylcarbodi-imide (925 mg, 4.5 mmol) were added. The mixture was kept at room temperature for 24 h, and the *product* was worked up as described above; yield 584 mg (71%); $[\alpha]_D^{20}$ −36.2° (*c* 0.7) (Found: C, 57.0; H, 6.5; N, 10.4. $C_{26}H_{34}N_4O_9$ requires C, 57.15; H, 6.25; N, 10.25%), $\lambda_{max.}$ 208, 256, and 276 nm (log ε 4.27, 4.28, and 4.22), $\lambda_{min.}$ 234 and 267 nm (log ε 3.87 and 4.20), $\nu_{max.}$ 3 448, 3 135, 3 003, 1 701br, 1 664, 1 577br, and 701 cm^{-1}; τ(CD_3OD) 1.85 (1 H, d, 6-H; $J_{6.5}$ 8.0 Hz), 4.09 (1 H, d, 5-H; $J_{5.6}$ 8.0 Hz), 6.29 and 6.34 (2 H, q, CH_2O; J_{Et} 7.0 Hz), and 8.75 and 8.93 (3 H, t, CH_3; J_{Et} 7.0 Hz).

5′-O-*Acetyl*-2′,3′-O-*isopropylidene*-2-N-(N-*t-butoxycarbonyl*-L-*phenylalanyl*)*isocytidine* (11).—The aminoacylisocytidine (5) (265 mg, 0.5 mmol) in anhydrous pyridine (5 ml) was acetylated with acetic anhydride (1 ml) at room temperature for 16 h and the product was worked up as for compound (2) to give a *foam* (240 mg, 84%), R_F *ca.* 0.7; $[\alpha]_D^{21}$ −26.3° (*c* 1.9 in CH_2Cl_2) (Found: C, 58.95; H, 6.65; N, 9.75. $C_{28}H_{36}N_4O_9$ requires C, 58.75; H, 6.35; N, 9.8%); $\lambda_{max.}$ 207, 255, 274, and 291sh nm (log ε 4.28, 4.26, 4.21, and 3.99) $\lambda_{min.}$ 233 and 266 nm (log ε 3.92 and 4.19); $\nu_{max.}$ 3 413, 2 994, 1 745, 1 704, 1 639, 1 577, and 702 cm^{-1}; τ 2.36 (1 H, d, 6-H; $J_{6.5}$ 8.3 Hz), 2.84 (5 H, s, aromatic), 4.18 (1 H, d, 5-H; $J_{5.6}$ 8.3 Hz), 7.99 (3 H, s, OAc), 8.38 and 8.64 (2 × 3 H, s, CMe_2), and 8.64 (9 H, s, Bu^t).

2-N-(N-*t-Butoxycarbonyl*-L-*phenylalanyl*)*isocytidine* (12).—To a suspension of isocytidine (100 mg, 0.41 mmol) in anhydrous dioxan (5 ml), *N*-t-butoxycarbonyl-L-phenyl-L-phenylalanine (136 mg, 0.5 mmol) and *NN′*-dicyclohexylcarbodi-imide (152 mg, 0.74 mmol) in dioxan (5 ml) and water (1 ml) were added. The mixture was stirred for 24 h, then more *NN′*-dicyclohexylcarbodi-imide (106 mg, 0.8 mmol) was added. The product was worked up as described above to give a *foam* (48 mg, 25%); $[\alpha]_D^{24}$ − 22.7° (*c* 0.6); dried at 80 °C and 10^{-5} mmHg (Found: C, 57.85; H, 6.3; N, 11.5. $C_{23}H_{30}N_4O_8$ requires C, 58.2; H, 6.35; N, 11.8%); $\lambda_{max.}$ 207, 257, and 273 nm (log ε 4.25, 4.27, and 4.20), $\lambda_{min.}$ 234 and 268 nm (log ε 3.91 and 4.20); $\nu_{max.}$ 3 509br, 3 030, 2 967, 1 695br, 1 639, 1 587br, and 702 cm^{-1}; τ(CD_3OD) 1.63 (1 H, d, 6-H; $J_{6.5}$ 8.3 Hz), 2.80 (5 H, s, aromatic), 4.09 (1 H, d, 5-H; $J_{5.6}$ 8.3 Hz), and 8.63 (9 H, s, Bu^t).

2′,3′-O-*Isopropylidene*-2-N-(N-*triphenylmethylglycyl*-*glycyl*)*isocytidine* (13).—To a solution of isocytidine (1) (70.7 mg, 0.25 mmol) in dimethylformamide (4 ml), the hydroxysuccinimido-ester of *N*-triphenylmethylglycylglycine [17] (237 mg, 0.5 mmol) was added. The mixture was heated at 95 °C for 4 h, then kept at room temperature for 24 h and evaporated to dryness. The residue was triturated with water. A solid separated which, washed with ether, yielded the *product* (13) (129 mg, 81%), m.p. 122—125 °C (from dichloromethane–n-hexane) (Found: C, 65.85; H, 6.2; N, 10.9. $C_{35}H_{37}N_5O_7$ requires C, 65.7; H, 5.85; N, 10.95%); $\lambda_{max.}$ 257 and 270sh nm (log ε 3.55 and 3.48), $\lambda_{min.}$ 241 nm (log ε 3.33), $\nu_{max.}$ 3 413, 3 086, 2 915, 1 695, 1 631, 1 580br, and 708 cm^{-1}.

2-N-(N-*Triphenylmethylglycylglycyl*)*isocytosine* (14).—To a solution of isocytosine (111 mg, 1 mmol) in dimethylformamide–dimethyl sulphoxide (8 ml; 1 : 1) the hydroxysuccinimido-ester of *N*-triphenylmethylglycylglycine [17] (471 mg, 1 mmol) in dimethylformamide (4 ml) was added. The *product* was worked up as for compound (13), purified on a silica gel plate (CH_2Cl_2–MeOH, 10 : 1), and crystallized from methanol; yield 380 mg (80%), m.p. 221—222 °C (from chloroform–n-hexane); dried at 80 °C and 10^{-5} mmHg (Found: C, 67.1; H, 5.5; N, 14.7. $C_{27}H_{25}N_5O_3$,-H_2O requires C, 66.8; H, 5.6; N, 14.45%), $\lambda_{max.}$ 220sh and 268 nm (log ε 4.45 and 3.92), $\lambda_{min.}$ 253 nm (log ε 3.66); $\nu_{max.}$ 3 436, 3 289, 3 115, 2 985, 1 685br, 1 621, 1 565, and 706 cm^{-1}.

2-N-*Glycylglycylisocytosine* (15).—A solution of 2-*N*-(*N*-triphenylmethylglycylglycyl)isocytosine (14) (121.5 mg, 0.25 mmol) in 50% acetic acid was heated at 95 °C for 10 min and then diluted with water (5 ml). The precipitate was filtered off and the filtrate evaporated to dryness to give the *product* (53 mg, 94%), m.p. 270—275 °C (decomp.) [from MeOH–H_2O (4 : 1)] (Found: C, 42.4; H, 5.0; N, 29.65. $C_8H_{11}N_5O_5$ requires C, 42.65; H, 4.9; N, 31.1%), $\nu_{max.}$ 3 125br, 1 667br, and 1603 cm^{-1}; τ(D_2O) 2.42 (1 H, d, 6-H; $J_{6.5}$ 7.0 Hz), 4.14 (1 H, d, 5-H; $J_{5.6}$ 7.0 Hz), and 6.0 (2 × 2 H, s, 2 × CH_2).

Acidic Hydrolysis of 2′,3′-O-*Isopropylidene*-2-N-(N-*t-butoxycarbonyl*-L-*phenylalanyl*)*isocytidine* (5).—(*a*) The 2-*N*-Aminoacylisocytidine (5) (400 mg, 0.76 mmol) dissolved in 98% trifluoroacetic acid (3.5 ml) was kept at −12 °C for 5 min and then the solution was evaporated to dryness. 2′,3′-O-*Isopropylidene*-2-N-L-*phenylalanylisocytidine trifluoroacetate salt* (16) separated on trituration with ether and methylene chloride (yield 265 mg, 65%); m.p. 173—175 °C (from methanol–ether); $[\alpha]_D^{22}$ −48.6° (*c* 0.78 in 50% EtOH) (Found: C, 50.8; H, 5.1; N, 10.6. $C_{21}H_{26}N_4O_6$,-CF_3CO_2H requires C, 50.75; H, 5.0; N, 10.3%); $\lambda_{max.}$ 210, 259, 281. and 314sh nm (log ε 4.49, 4.34, 4.36, and 3.85), $\lambda_{min.}$ 239 and 266 nm (log ε 4.11 and 4.31); $\nu_{max.}$ 3 521, 3 145br, 2 674, 1 695sh, 1 678, 1 634, 1 587br, and 706 cm^{-1}; τ(CD_3OD) 1.80 (1 H, d, 6-H: $J_{6.5}$ 8.3 Hz), 2.67 (5 H, s, aromatic), 4.00 (1 H, d, 5-H; $J_{5.6}$ 8.3 Hz), and 8.46 and 8.71 (2 × 3 H, s, CMe_2).

(*b*) The chromatographically homogeneous compound (5) (400 mg, 0.76 mmol) was treated with 98% trifluoroacetic acid (3.5 ml) at 23 °C for 5 min and then the mixture was evaporated to dryness. Preparative t.l.c. [development in methylene chloride–methanol (10 : 1)] separated (i) starting material (41 mg, 10.2%), R_F *ca.* 0.6; (ii) a foam identified as 2-N-L-*phenylalanylisocytosine* (17) (107 mg, 55.4%), R_F *ca.* 0.25 (Found: C, 60.40; H, 5.75; N, 21.7. $C_{13}H_{14}$-N_4O_2 requires C, 60.45; H, 5.45; N, 21.3%); τ 2.42 (1 H, d, 6-H; $J_{6.5}$ 6.8 Hz), 2.78 (5 H, s, aromatic), and 4.30 (1 H, d, 5-H; $J_{5.6}$ 6.8 Hz); (iii) 2′,3′-O-*isopropylidene*-5′-O-(N-*t-butoxycarbonyl*-L-*phenylalanyl*)*isocytidine* (18) (31 mg, 7.8%); R_F *ca.* 0.5; τ(CD_3OD) 2.39 (1 H, d, 6-H; $J_{6.5}$ 7.8 Hz), 2.75 (5 H, s, aromatic), 4.05 (1 H, d, 5-H; $J_{5.6}$ 7.8 Hz), 8.41 and 8.64 (2 × 3 H, s, CMe_2), and 8.63 (9 H, s, Bu^t); and (iv) a minor hygroscopic component, tentatively identified as 2′,3′-*O*-isopropylidene-5′-*O*-L-phenylalanylisocytidine trifluoroacetate salt (19), τ(CD_3OD) 2.31 (1 H, d, 6-H; $J_{6.5}$ 7.8 Hz), 2.78 (5 H, s, aromatic), 4.22 (1 H, d, 5-H; $J_{5.6}$ 7.8 Hz), and 8.42 and 8.66 (2 × 3 H, s, CMe_2).

2′,3′-O-*Ethoxymethylene*-5,6-*dihydroisocytidine* (20).—A solution of 2′,3′-*O*-ethoxymethyleneisocytidine (9) (100 mg, 0.3 mmol) in methanol (15 ml) was hydrogenated over 5% rhodium–carbon (90 mg) at 55 lb in^{-2} for 5 h. Work-up as

for 2′,3′-*O*-isopropylidene-5,6-dihydrouridine [22] gave a *foam* in quantitative yield; $[\alpha]_D^{24}$ −11° (*c* 0.8) (Found: C, 45.2; H, 6.25; N, 13.05. $C_{12}H_{19}N_3O_6,H_2O$ requires C, 45.15; H, 6.65; N, 13.15%), $\lambda_{max.}$ 207 and 239 nm (log ε 4.12 and 4.14), $\lambda_{max.}$ 222 nm (log ε 3.94); $\nu_{max.}$ 3 448br, 2 976, 1 736, 1 645br, and 1 608 cm^{-1}; τ(CD_3OD) 6.33 and 6.38 (2 H, q, CH_2O; J_{Et} 7.0 Hz), 6.37 (2 H, t, 6-H_2; $J_{6.5}$ 6.7 Hz), 7.45 (2 H, t, 5-H_2; $J_{5.6}$ 6.7 Hz), and 8.76 and 8.82 (3 H, t, CH_3; J_{Et} 7.0 Hz).

2-N-(*Glycylglycyl*)-5,6-*dihydroisocytosine* (21).—A solution of 2-*N*-(glycylglycyl)isocytosine (40 mg, 0.178 mmol) in distilled water (2 ml) was hydrogenated over 5% rhodium–carbon (40 mg) at 50 lb in^{-2} for 16 h as described above to give the *product* (21) (33.5 mg, 83%), m.p. 290—300 °C (decomp.) (from 80% methanol) (Found: C, 42.0: H, 5.85; N, 30.45. $C_8H_{13}N_5O_3$ requires C, 42.3; H, 5.75; N, 30.8%), $\nu_{max.}$ 3 205, 3 030br, 2 865, and 1 686 cm^{-1}.

5,6-*Dihydro*-2′,3′-O-*isopropylidene*-2-N-(N-*t*-*butoxycarbonylglycyl*)*isocytidine* (22).—Compound (7) (110 mg, 0.25 mmol), hydrogenated as described above, gave a *foam* (100%); $[\alpha]_D^{22}$ −47.8° (*c* 0.56) (Found: C, 51.7; H, 7.1; N, 12.45. $C_{19}H_{30}N_4O_8$ requires C, 51.55; H, 6.85; N, 12.65%); $\lambda_{max.}$ 234sh and 259 nm (log ε 4.06 and 4.21), $\lambda_{max.}$ 207 nm (log ε 3.82); $\nu_{max.}$ 3 412br, 2 967, 2 907, 1 704br, and 1 582br cm^{-1}; τ 6.33 (2 H, t, 6-H_2; $J_{6.5}$ 6.7 Hz), 7.35 (2 H, t, 5-H_2; $J_{5.6}$ 6.7 Hz), 8.40 and 8.62 (2 × 3 H, s, CMe_2), and 8.57 (9 H, s, Bu^t).

5,6-*Dihydro*-2′,3′-O-*isopropylidene*-2-N-(N-*t*-*butoxycarbonyl*-L-β-*cyclohexylalanyl*)*isocytidine* (23).—Compound (5) (106 mg, 0.2 mmol), hydrogenated as described above, yielded the crystalline *product* (23) (99%), m.p. 161—163 °C (from methylene chloride–n-hexane); $[\alpha]_D^{22}$ −50.7° (*c* 0.88) (Found: C, 57.8; H, 7.8; N, 10.1. $C_{26}H_{42}N_4O_8$ requires C, 57.95; H, 7.85; N, 10.4%), $\lambda_{max.}$ 227 and 260 nm (log ε 4.03 and 4.25), $\lambda_{min.}$ 206 and 238 nm (log ε 3.82 and 3.98); $\nu_{max.}$ 3 484, 3 448, 2 994, 2 941, 2 865, 1 718, 1 672, and 1 597br cm^{-1}; τ 6.30 (2 H, t, 6-H_2; $J_{6.5}$ Hz), 7.30 (2 H, t, 5-H_2; $J_{5.6}$ 6.5 Hz), 8.40 and 8.62 (2 × 3 H, s, CMe_2), 8.57 (9 H, s, Bu^t), and 8.05—9.0 (11 H, m, cyclohexyl).

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