Synthesis and Antileukemic Activity of Chymotrypsin-Activated Derivatives of 3′-Amino-2′,3′-dideoxycytidine. (Synthetic Nucleosides and Nucleotides. XXXIII)[1)]

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3′-Amino-2′,3′-dideoxycytidine (8) was directly synthesized from 2′-deoxycytidine. 2′,3′-Dideoxy-3′-(*N*-acyl-L-phenylalanylamino)cytidines (acyl = butoxycarbonyl (9a), acetyl (9b), benzoyl (9c), and *n*-hexanoyl (9d)) were synthesized as chymotrypsin-activated prodrugs of 8. This N-protection was required for activation by chymotrypsin to 8. *In vitro*, compound 8 showed high cytotoxic activity against P388 cells, but the prodrugs 9a—d were ineffective. *In vivo*, however, these prodrugs showed much higher activity than 8 in mice bearing P388 cells.

Key words 3′-amino-2′,3′-dideoxycytidine; phenylalanine; chymotrypsin; enzymatic activation; prodrug; P388

A high and well-maintained drug concentration and specific activation in the body are ideal characteristics for antimetabolites in cancer chemotherapy. However, rapid enzymatic degradation and/or undesirably rapid elimination are observed in the case of the clinically indispensable agent 1-β-D-arabinofuranosylcytosine (AraC). Though many derivatives of AraC have been synthesized in attempts to improve the kinetic properties, and some of them have been developed for clinical use, their activation to the parent drug is dependent on spontaneous hydrolysis and/or non-specific enzymatic reactions.[2-7)] For better kinetic profiles or specific activation of prodrugs, a novel molecular design focusing on enzyme specificity to substrate (prodrug) has been desired. Part of our program on the design and synthesis of nucleoside analogues that exhibit antitumor activity *in vivo* is now focused on the synthesis of chymotrypsin-activatable derivatives of 2′,3′-dideoxy-3′-aminocytidine (**8**). Some proteases are known to be tumor-associated,[8)] and plasmin-activated prodrugs were reported to be potent anticancer agents.[9)] Synthesis and some biological activities of compound **8** and related compounds have been reported previously.[10)] Such compounds show cytotoxic activities *in vitro*, but there are few *in vivo* data on L1210 in mice.[11)] We describe here the convenient direct synthesis of compound **8** from 2′-deoxycytidine (**1**) and derivatization of **8** to various 3′-*N*-acylated *N*-L-phenylalanylamido-2′,3′-dideoxycytidines (**9a—d**, Fig. 1). The compounds thus obtained have potent antileukemic activities on P388 leukemia in mice (up to 171% ILS).

Chemistry

For the present study, naturally occurring 2′-deoxycytidine (**1**) was selected as a precursor for the required key intermediate (Chart 1). First, compound **1** was reacted with trityl chloride to afford 5′-*O*-trityl-2′-deoxycytidine (**2**), followed by treatment with methanesulfonyl chloride in anhydrous pyridine at low temperature (−15 °C) to give 5′-*O*-trityl-3′-*O*-methanesulfonyl-2′-deoxycytidine (**3**) in good yield. Treatment of **3** with triethylamine in 30% aqueous dioxane at 50 °C afforded to crystalline 1-(5-*O*-trityl-2-deoxy-1-β-D-xylofuranosyl)cytosine (**4**) *via* the 2,3′-anhydro intermediate (**3a**). Compound **4** was methanesulfonylated again in pyridine under similar reaction conditions to give 1-(5-*O*-trityl-2-deoxy-3-*O*-methanesulfonyl-1-β-D-xylofuranosyl)cytosine (**5**) in satisfactory yield. Treatment of **5** with lithium azide in *N*,*N*-dimethylformamide (DMF) under a nitrogen atmosphere at 70 °C gave 5′-*O*-trityl-3′-azido-2′,3′-dideoxycytidine (**6**) in excellent yield. Reduction of compound **6** was performed with hydrogen sulfide in pyridine at room temperature to give the corresponding 3′-amino derivative (**7**). Deblocking of the trityl group in **7** afforded the key intermediate (**8**).

For the synthesis of *N*-phenylalanyl derivatives, the pentachlorophenyl ester of *N*-*tert*-butoxycarbonyl (*tert*-BOC)-L-phenylalanine was allowed to react with **7** in DMF in the presence of triethylamine to give 2′,3′-dideoxy-3′-(*N*-*tert*-butoxycarbonyl-L-phenylalanylamino)cytidine (**9a**) in good yield. Treatment of trifluoroacetic acid with **9a** gave 2′,3′-dideoxy-3′-L-phenylalanylaminocytidine (**9**). Derivatization of **9** to its *N*-acetylated counterpart (**9b**) was achieved by treatment with acetic anhydride using a conventional procedure. Reaction of *N*-acylated-L-phenylalanine esters with **8** gave the *N*-benzoyl (**9c**) and *N*-*n*-hexanoyl (**9d**) counterparts, respectively.

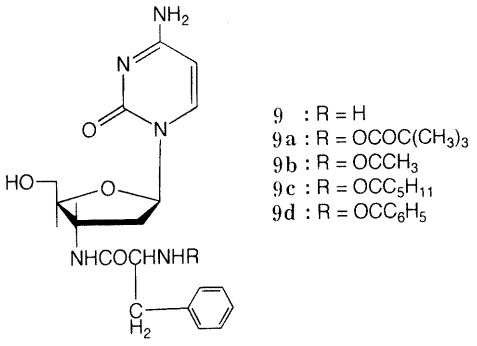

Fig. 1. Structures of *N*-Acyl-L-phenylalanyl Prodrugs of 3′-Amino-2′,3′-dideoxycytidine

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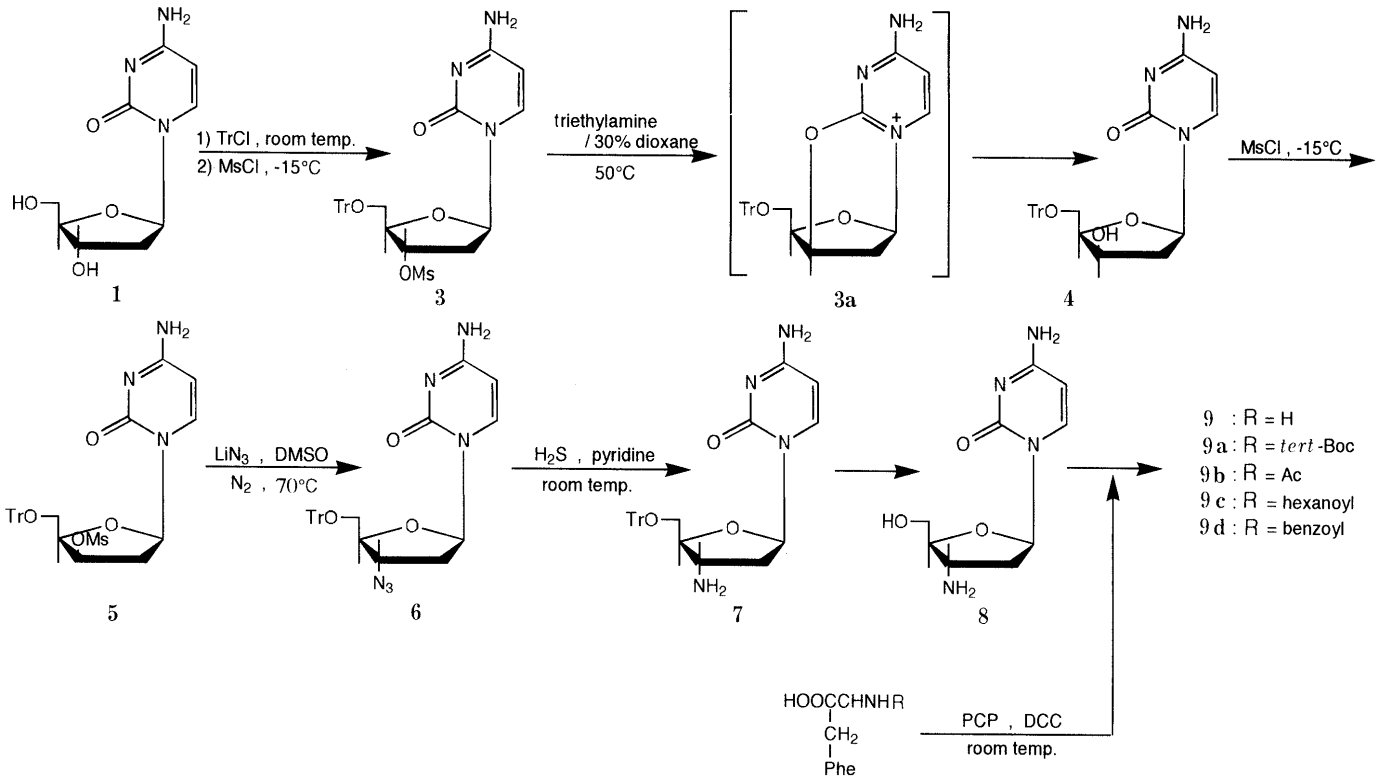

Chart 1

Biological Evaluations and Discussion

The enzymatic hydrolysis of the compounds (**9a—d**) by chymotrypsin from bovine pancreas resulted in the formation of *N*-acyl-L-phenylalanine and 3′-amino-2′,3′-dideoxycytidine (**8**) in the ratio of 1 : 1. However, the hydrolysis of 2′,3′-dideoxy-3′-*N*-L-phenylalanylaminocytidine (**9**) was not catalyzed by this endopeptidase, chymotrypsin. Typical concentration–time profiles of **9b** and regenerated **8** are shown in Fig. 2. Since these prodrugs were stable in rat and human plasma (data not shown), parenterally administered prodrugs may be activated in tumor and other chymotrypsin-associated tissues, such as liver.

Antitumor activity against P388 cells was evaluated both *in vitro* and *in vivo*. *In vitro*, the key compound **8** showed high activity ($IC_{50} = 0.32\ \mu M$), though the prodrugs (**9a—d**) were ineffective ($IC_{50} > 50\ \mu M$). This may be attributable to the lack of activation enzyme (chymotrypsin) in the cultured cells. *In vivo*, however, the prodrugs (**9a—d**) administered intraperitoneally once a day from 1 to 5 d after tumor cell inoculation showed higher activity and better therapeutic index than the key compound (Table I). The maximal increase-in-lifespan (ILS) of **8** was 108 at a dose of 300 mg/kg/d. The maximal ILS values of **9a**, **9b**, **9c**, and **9d** were improved to 135, 120, 131 and 171, respectively. In paticular, the benzoylated compound (**9c**) showed this ILS at a lower optimal dose of 100 mg/kg/d. The therapeutic index, *i.e.*, optimal dose divided by the dose giving an ILS of 30% (ILS_{max}/ILS_{30}), of **9c** and **9d** was remarkably improved from that of **8** (see the footnote in Table I).

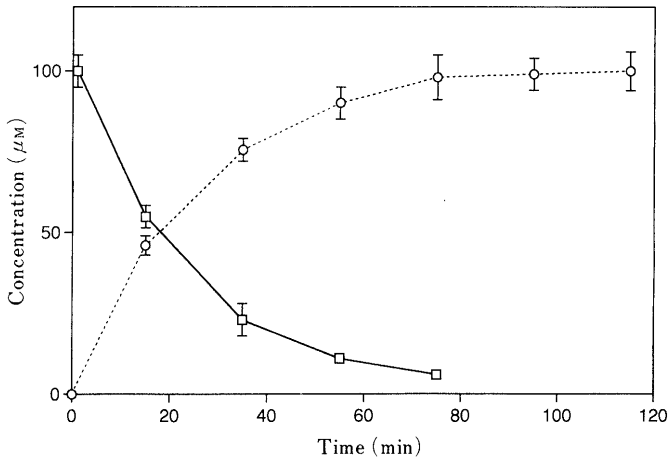

Fig. 2. Time Course of Disappearance of the *N*-Acetyl Derivative **9b** and Appearance of Its Hydrolysis Product **8** in the Presence of Chymotrypsin (40 units/ml, pH 7.4) at 37 °C

Averages of three experiments. □, **9b**; ○, **8**. The *N*-free derivative (**9**) was not detected.

Experimental

Biological Test Procedures Enzymatic activation of the prodrugs was studied as described previously.[12] Mouse P388 cells were maintained as suspension cultures in serum free medium (ASF-MED104, Ajinomoto, Japan) at 37 °C in a humidified atmosphere of 5% CO_2–95% air. Under these conditions, the generation time for P388 cells is approximately 16 h. Each compound at the given concentration was added to P388 cells (2×10^4 cells/l). Growth-inhibitory activity was determined by measuring the number of cells. Viable cell count was estimated by means of the trypan blue exclusion test. *In vivo* evaluation of antitumor activity was done by the method described previously.[13]

Chemistry Melting points were determined on a Yanaco MP-3 apparatus and are uncorrected. Ultraviolet (UV) spectra were recorded on a Shimadzu UV-300 recording spectrophotometer. Mass spectra (MS) were measured on a Hitachi RMU-6E mass spectrometer. Nuclear

TABLE I. Antitumor Activity of 3′-Amino-2′,3′-dideoxycytidine (**8**) and Its Prodrugs (**9a—d**) in Mice Bearing P388 Cells

Compound	Dose[a]	MST±S.E.[b]	Control	ILS[c]	Weight[d]
Control A	—	9.0±0.8	—	—	+3.6
Control B	—	10.0±0.0	—	—	+2.9
Control C	—	9.8±0.4	—	—	+2.6
8	10	9.3±0.7	A	3	+2.0
	30	10.5±0.8	A	16	+1.4
	100	14.3±0.5	A	59	+0.6
	300[e]	18.6±0.5	A	108	−0.2
	350	14.4±0.5	B	43	−3.6
	400	12.5±0.5	B	25	−5.9
9a	10	9.2±0.4	A	2	+1.1
	30	10.0±0.8	A	11	+1.5
	100	13.2±0.4	A	47	+0.8
	300	16.6±0.5	A	85	−1.2
	350[e]	23.5±0.5	B	135	−4.9
	400	15.3±0.4	B	53	−5.5
9b	10	12.4±1.5	C	27	+3.2
	30	12.2±1.5	C	24	+3.5
	100	20.0±1.3	C	104	+1.2
	300[e]	21.6±0.5	C	120	−0.4
9c	10	14.2±1.0	C	45	+3.8
	30	15.4±0.5	C	57	+3.2
	100	21.6±2.0	C	120	+1.9
	300[e]	22.6±0.8	C	131	+0.2
9d	10	16.2±1.3	C	57	+3.2
	30	19.6±0.5	C	100	+3.7
	100[e]	26.6±1.4	C	171	+2.4
	300	24.4±1.2	C	149	+0.1

a) mg/kg/d. Doses of **9a—d** were calculated as compound **8**. *b*) Mean survival time (d). *c*) Increase in life span (%). *d*) Weight change, 0—5 d, g/mouse. *e*) ILS_{max}, optimal dose. Therapeutic ratios calculated from ILS_{max} and ILS_{30} for **8**, **9a**, **9b**, **9c**, and **9d** were 5.7, 5.0, 10.0, 45.0, and 18.8, respectively.

magnetic resonance (NMR) spectra were obtained on a Hitachi R20B high-resolution NMR spectrometer using tetramethylsilane as the internal standard. Circular dichroism (CD) spectra were measured on a JASCO model 20 automatic recording spectropolarimeter.

1-(2-Deoxy-5-*O*-trityl-1-β-D-xylofuranosyl)cytosine (4) 2′-Deoxycytidine (**1**), 10 g (44 mmol) in 200 ml of anhydrous pyridine was treated with trityl chloride (13.3 g, 48 mmol, 1.1 eq) and stirred at room temperature for 4 d. The solution was evaporated to dryness and the residue was partitioned between chloroform and distilled water. The chloroform layer was dried over magnesium sulfate, concentrated to a small volume (*ca.* 30 ml) and purified by silica gel column chromatography (300 g, chloroform–ethanol, 9 : 1). The combined eluates were evaporated to dryness and crystallized from acetone–ethanol. Yield, 16.1 g (78%), mp 223—225 °C.

The resulting 2′-deoxy-5′-*O*-tritylcytidine (**2**), 10 g (21.3 mmol) in anhydrous pyridine (200 ml) was treated dropwise with methanesulfonyl chloride (2.5 ml, 1.5 eq) under cooling (−15 °C) and stirring. The mixture was further stirred under 0 °C for 20 h, then treated with methanol (*ca.* 10 ml), and evaporated. The residue was chromatographed on silica gel (80 g) with chloroform–ethanol (9 : 1). 2′-deoxy-3′-*O*-methanesulfonyl-5′-*O*-tritylcytidine (**3**) was obtained as a thin-layer-chromatographically homogeneous foam (11 g, 94%). This foam was dissolved in 30% aqueous dioxane (80 ml), then the solution was mixed with 40 ml of triethylamine and the whole was heated at 50 °C for 24 h under stirring. After cooling to room temperature, the resulting precipitates were collected by filtration and washed with distilled water, ethanol and ether. Yield 7.0 g (70%). A sample was crystallized from boiling ethanol to give fine crystals, mp 226—228 °C. UV λ_{max}^{MeOH} nm: 270. ^{1}H-NMR (Me_2SO-d_6) δ: 3.1—3.5 (4H, m, C(5′)-H, C(2′)-H), 3.9—4.2 (2H, m, C(3)-H, C(4′)-H), 5.12 (1H, d, C(3′)-OH), 5.62 (1H, d, C(5)-H), 6.05 (1H, d, C(1′)-H), 7.06 (2H, s, N(4)-H), 7.3—7.5 (15H, m, trityl), 7.64 (1H, d, C(6)-H). *Anal.* Calcd for $C_{28}H_{27}N_3O_4 \cdot 0.75C_2H_5OH$: C, 70.29; H, 6.30; N, 8.34. Found: C, 70.16; H, 6.21; N, 8.58.

1-(2-Deoxy-3-*O*-methanesulfonyl-5-*O*-trityl-1-β-D-xylofuranosyl)cytosine (5) Methanesulfonyl chloride (1.2 ml, 15.5 mmol, 1.5 eq) was added to compound **4** (5 g, 10.6 mmol) in anhydrous pyridine (100 ml) under cooling at −20 °C and the mixture was stirred at 0 °C for 24 h. Methanol (10 ml) was added, and the reaction mixture was stirred for another 30 min, then concentrated. The residue was treated with saturated sodium hydrogen carbonate and chloroform (1 : 1, 200 ml). After vigorous shaking, the organic layer was separated, washed with distilled water, dried, and evaporated under reduced pressure. The residue was dissolved in chloroform (30 ml) and this solution was applied to a column of silica gel (100 g). Elution was performed with chloroform–ethanol (9 : 1). The fractions containing the desired nucleoside were combined and evaporated to dryness. The residue was crystallized from 90% aqueous ethanol to give fine needles, 3.6 g (62%), mp 205—206 °C. UV λ_{max}^{EtOH} nm: 269. ^{1}H-NMR (Me_2SO-d_6) δ: 2.0—2.9 (2H, m, C(2′)-H), 3.0 (3H, s, $-SO_2-CH_3$), 3.1—3.6 (2H, m, C(5′)-H), 4.2—4.6 (1H, m, C(4′)-H), 5.29 (1H, t, C(3′)-H), 6.11 (1H, d, C(5)-H), 7.16 (2H, s, N(4)-H), 7.2—7.4 (16H, m, C(6)-H, trityl). *Anal.* Calcd for $C_{29}H_{29}N_3O_6S \cdot 1/2H_2O$: C, 62.57; H, 5.43; N, 7.55; S, 5.76. Found: C, 62.77; H, 5.20; N, 7.67; S, 5.85.

3′-Azido-2′,3′-dideoxy-5′-*O*-tritylcytidine (6) A solution of **5** (1.5 g 2.74 mmol) in DMF (15 ml) was treated with lithium azide (350 mg) and the mixture was stirred at 70 °C under a nitrogen atmosphere for 3 h, then evaporated to dryness under diminished pressure. The residue was partitioned between chloroform and distilled water, and the organic layer was then dried over magnesium sulfate and evaporated. The residue was crystallized from boiling 90% aqueous ethanol to give fine needles, 1.3 g (96%), mp 142—144 °C. IR ν_{max}^{KBr} cm^{-1}: 2070 (N_3). ^{1}H-NMR (Me_2SO-d_6) δ: 2.37 (2H, t, C(2′)-H), 3.2—3.4 (2H, m, C(2)-H, C(5′)-H), 3.7—4.0 (1H, m, C(4′)-H), 4.5 (1H, q, C(3′)-H), 5.59 (1H, d, C(5)-H), 6.1 (1H, t, C(1′)-H), 7.17 (2H, s, N(4)-H), 7.2—7.5 (15H, m, trityl), 7.69 (1H, d, C(6)-H). *Anal.* Calcd for $C_{28}H_{26}N_6O_3 \cdot 1/4H_2O$: C, 67.64; H, 5.48; N, 16.61. Found: C, 67.38; H, 5.23; N, 15.46.

3′-Amino-2′,3′-dideoxy-5′-*O*-tritylcytidine (7) Compound **6** (3.3 g, 6.6 mmol) in 50% aqueous pyridine (30 ml) was treated with hydrogen sulfide by passing the gas through it for 1 h. The solution was neutralized with 2 N acetic acid and then evaporated to dryness. The residue was partitioned between chloroform and distilled water (1 : 1, 100 ml). After vigorous shaking, the organic layer was dried over magnesium sulfate and concentrated to dryness. The residue was purified by column chromatography on silica gel (50 g, chloroform–ethanol, 9 : 1 and 4 : 1). The fractions containing **7** were combined and evaporated, and the residue was crystallized from 90% aqueous ethanol. 2.1 g (68%), mp 242—244 °C. ^{1}H-NMR (Me_2SO-d_6) δ: 2.07 (2H, t, C(2′)-H), 3.2—3.8 (4H, m, C(3′)-H, C(4′)-H, C(5′)-H), 4.0—4.4 (2H, s, N(3′)-H), 5.53 (1H, d, C(5)-H), 6.09 (1H, t, C(1′)-H), 7.05 (2H, s, N(4)-H), 7.1—7.5 (15H, m, trityl), 7.72 (1H, d, C(6)-H). *Anal.* Calcd for $C_{28}H_{28}N_4O_3 \cdot H_2O$: C, 69.12; H, 6.21; N, 11.51. Found: C, 69.36; H, 6.03; N, 11.48.

3′-Amino-2′,3′-dideoxycytidine Dihydrogen Chloride (8) Method A: Compound **7** (940 g) in 80% acetic acid (10 ml) was heated at 100 °C for 20 min under mechanical stirring. After cooling to room temperature, the precipitated triphenyl carbinol was filtered off and the filtrate was concentrated to dryness. The residue was partitioned between diethyl ether and distilled water. The aqueous layer was evaporated to dryness and the residue was treated with ethanolic hydrogen chloride. The solution was evaporated again and the residue was crystallized from absolute ethanol to give fine needles, 200 mg (38%), mp 190—192 °C, ^{1}H-NMR (Me_2SO-d_6) δ: 2.0—2.4 (2H, m, C(2′)-H), 3.6—4.1 (5H, m, C(4′)-H, C(5′)-H, N(3′)-H), 4.14 (1H, d, C(3′)-H), 5.1—5.6 (1H, br, C(5′)-OH), 6.14 (1H, d, C(5)-H), 6.25 (1H, t, C (1′)-H), 8.18 (1H, d, C(6)-H), 8.4—8.8 (4H, br, N(4)-H, HCl). *Anal.* Calcd for $C_9H_{14}N_4O_3 \cdot 2HCl$: C, 36.13; H, 5.39; Cl, 23.70; N, 18.73. Found: C, 36.16; H, 5.26; Cl, 23.61; N, 18.47.

Method B: Compound **6** (300 mg) was dissolved in 80% acetic acid (5 ml) and the solution was heated at 100 °C for 20 min. After cooling, precipitated triphenyl carbinol was filtered off and the filtrate was evaporated. The residue was purified by column chromatography on silica gel (5 g). Elution was performed with chloroform–ethanol (4 : 1), then the fractions containing azido-nucleoside were combined and evaporated under diminished pressure. The residue was crystallized from boiling ethanol to give 100 mg (65%) of 3′-azido-2′,3′-dideoxycytidine as fine needles, mp 144—146 °C (lit.[14] mp 143 °C). This compound was treated with hydrogen sulfide in pyridine to afford the 3′-amino compound after conventional purification as described above. Final yield of **8** was 85 mg. Comparison of UV and NMR spectra and mixed melting point test showed that this specimen was identical with the sample obtained by method A.

2′,3′-Dideoxy-3′-(*N*-*tert*-butoxycarbonyl-L-phenylalanylamino)cytidine

(9a) Triethylamine (300 μl, 1.1 eq) and *N-tert*-butoxycarbonyl-L-phenylalanine pentachlorophenyl ester (1.1 g, 2.2 mmol) were added to the aminonucleoside **8** (450 mg, 1.99 mmol) in DMF (5 ml) and the resulting solution was stirred at room temperature for 24 h. The reaction mixture was concentrated under reduced pressure and the residue was partitioned between ethyl acetate and distilled water. The organic layer was evaporated and the residue was purified by silica gel column chromatography (30 g, chloroform–ethanol, 9 : 1). The fractions containing **9a** were combined and evaporated *in vacuo*. The residue was crystallized from ethanol containing a small amount of diethyl ether. 600 mg (63%), mp 163—165 °C. ^{1}H-NMR (Me_2SO-d_6) δ: 1.39 (9H, s, $CO(\underline{Me})_3$), 2.0—2.2 (2H, m, C(2′)-H), 2.6—3.2 (3H, m, CH–$C\underline{H}_2$–Phe), 3.4—3.7 (2H, m, N(3′)-H, N$\underline{H}$-*tert*-Boc), 3.9—4.4 (3H, m, C(4′)-H, C(5′)-H), 4.98 (1H, t, C(3′)-H), 5.73 (1H, d, C(5′)-OH), 6.16 (1H, t, C(1′)-H), 6.86 (1H, d, C(5)-H), 7.12 (2H, s, N(4)-H), 7.2—7.4 (5H, s, $-C_6\underline{H}_5$), 8.32 (1H, d, C(6)-H). *Anal.* Calcd for $C_{23}H_{31}N_5O_6$: C, 58.34; H, 6.60; N, 14.79. Found: C, 58.11; H, 6.41; N, 14.51.

2′,3′-Dideoxy-3′-(*N*-L-phenylalanylamino)cytidine (9) Compound **9a** (400 mg, 0.84 mmol) was dissolved in trifluoro acetic acid (5 ml) and the solution was stirred at room temperature for 10 min, then evaporated under reduced pressure. The residue was partitioned between chloroform and distilled water, and the aqueous layer was evaporated to dryness. The residue was dissolved in a small amount of methanol and a powder was precipitated by adding ethyl ether, 120 mg (38%), mp 255—258 °C, UV λ_{max}^{MeOH} nm: 271. *Anal.* Calcd for $C_{18}H_{23}N_5O_4 \cdot H_2O$: C, 55.23; H, 6.44; N, 17.89. Found: C, 55.33; H, 6.40; N, 17.77.

2′,3′-Dideoxy-3′-(*N*-acetyl-L-phenylalanylamino)cytidine (9b) A solution of **9** (100 mg, 0.27 mmol) in anhydrous pyridine (5 ml) was treated with acetic anhydride (0.05 ml) at 0 °C and the mixture was stirred at room temperature for 24 h, then evaporated *in vacuo*. The residue was partitioned between chloroform and distilled water. The organic layer was dried over magnesium sulfate and evaporated to dryness. The residue was crystallized from ethanol containing a small amount of ethyl ether, 83 mg (75%), mp 168—171 °C. UV λ_{max}^{MeOH} nm: 271. ^{1}H-NMR (Me_2SO-d_6) δ: 2.0—2.2 (2H, m, C(2′)-H), 3.3 (3H, s, CO$\underline{Me}$), 3.6 (2H, dd, CH–$C\underline{H}_2$–), 3.9—4.4 (3H, m, C(4′)-H, C(5′)-H), 4.5 (1H, m, –C$\underline{H}$–CH_2–), 4.94 (1H, br, C(5′)–H), 4.98 (1H, t, C(3′)-H), 5.69 (1H, d, C(5)-H), 6.16 (1H, t, C(1′)-H), 7.06 (2H, d, N(4)-H), 7.1—7.3 (5H, m, $-C_6\underline{H}_5$), 7.87 (1H, d, C(6)-H), 8.4—8.6 (2H, m, N(3′)-H, N$\underline{H}$-COMe).

2′,3′-Dideoxy-3′-(*N*-hexanoyl-L-phenylalanylamino)cytidine (9c) Triethylamine (300 μl, 1.1 eq) and *N*-hexanoyl-L-phenylalanine pentachlorophenyl ester (1.5 g, 1.5 eq) were added to a solution of **8** (450 mg, 1.99 mmol) in DMF (5 ml), and the resulting solution was stirred at room temperature for 50 h, then concentrated under reduced pressure. The residue was partitioned between ethyl acetate and distilled water. The organic layer was evaporated and the residue was purified by silica gel column chromatography (30 g, chloroform–ethanol, 9 : 1). The fractions containing **9c** were combined and evaporated *in vacuo*. The residue was crystallized from ethanol containing a small amount of diethyl ether. 752 mg (79%), mp 66—67 °C. UV λ_{max}^{MeOH} nm: 271. ^{1}H-NMR ($CDCl_3$) δ: 0.8—0.9 (3H, t, CH_2-$\underline{Me}$), 1.1—1.35 (6H, m, $COCH_2$–$(C\underline{H}_2)_3$-Me), 1.5—1.6 (2H, t, –$COC\underline{H}_2$–), 2.0—2.2 (2H, m, C(2′)-H), 3.6 (2H, dd, –CH–$C\underline{H}_2$–Phe), 3.9—4.4 (3H, m, C(4′)-H, C(5′)-H), 4.5 (1H, m, –C$\underline{H}$–CH_2–Phe), 4.94 (1H, br, C(5′)-OH), 4.98 (1H, t, C(3′)-H), 5.69 (1H, d, C(5)-H), 6.16 (1H, t, C(1′)-H), 7.06 (2H, d, N(4)-H), 7.1—7.3 (5H, m, $-C_6\underline{H}_5$), 7.87 (1H, d, C(6)-H), 8.4—8.6 (2H, m, N(3′)-H, N$\underline{H}$-COC_5H_{11}).

2′,3′-Dideoxy-3′-(*N*-benzoyl-L-phenylalanylamino)cytidine (9d) Triethylamine (300 μl, 1.1 eq) and *N*-benzoyl-L-phenylalanine pentachlorophenyl ester (1.5 g, 1.5 eq) were added to a solution of **8** (450 mg, 1.99 mmol) in DMF (5 ml), and the resulting solution was stirred at room temperature for 50 h, then concentrated under reduced pressure. The residue was partitioned between ethyl acetate and distilled water. The organic layer was evaporated and the residue was purified by silica gel column chromatography (30 g, chloroform–ethanol, 9 : 1). The fractions containing **9c** were combined and evaporated *in vacuo*. The residue was crystallized from ethanol containing a small amount of diethyl ether, 840 mg (88%), mp 75—77 °C. UV λ_{max}^{MeOH} nm: 271. ^{1}H-NMR ($CDCl_3$) δ: 2.0—2.2 (2H, m, C(2′)-H), 3.6 (2H, dd, CH–$C\underline{H}_2$–Phe), 3.9—4.4 (3H, m, C(4′)-H, C(5′)-H), 4.5 (1H, m, –C$\underline{H}$–CH_2–Phe), 4.94 (1H, br, C(5′)-OH), 4.98 (1H, t, C(3′)-H), 5.69 (1H, d, C(5)-H), 6.16 (1H, t, C(1′)-H), 7.06 (2H, d, N(4)-H), 7.1—7.3 (5H, m, $-CH_2-C_6\underline{H}_5$), 7.4—7.53 (5H, m, CO–$C_6\underline{H}_5$), 7.87 (1H, d, C(6)-H), 8.4—8.6 (2H, m, N(3′)-H, N$\underline{H}$-benzoyl).

References and Notes

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