[Chem. Pharm. Bull. 36(9)3253—3256(1988)]

Syntheses of N^4,2′,3′,5′-Tetraacylcytidines from N^4-Acylcytosines, *via* Condensation with Tetraacylribose and Transribosylation with Acylated Purine Nucleosides

YOSHIHIRO SUGIURA,* SYUICHI FURUYA, and YOSHIYASU FURUKAWA

Chemistry Laboratories, Central Research Division, Takeda Chemical Industries, Ltd., Jusohonmachi, Yodogawa-ku, Osaka 532, Japan

(Received January 27, 1988)

N^4-Isobutyryl- and -(2-ethylhexanoyl)cytosines (Ia, b) were synthesized by acylation of cytosine. N^4,2′,3′,5′-Tetraacylcytidines (III) were synthesized by two methods involving stannic chloride catalysis. One involves condensation of N^4-acylcytosines (I) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (II), and the other involves transribosylation between N^4-acylcytosines and acyl-inosines or -guanosines (IVa—d). N^4-Octanoyl-2′,3′,5′-triacetylcytidine (IIIf) was converted into cytidine by deacylation in a good yield.

Keywords——N^4-acylcytosine; acylribose; acylinosine; acylguanosine; stannic chloride; N^4,2′,3′,5′-tetraacylcytidine; condensation; transribosylation; synthesis

Although the Lewis acid-catalyzed silyl-Hilbert–Johnson method is widely used for the synthesis of pyrimidine nucleosides, it has a drawback in that the sensitivity of silylated pyrimidines to water necessitates the strict exclusion of moisture.[1,2] We have developed industrially feasible methods for the synthesis of cytidine, which is a key intermediate in the synthesis of some medicines.

Condensation of N^4-Acylcytosines (I) and 1-*O*-Acetyl 2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (II)

We have previously reported the synthesis of purine nucleosides by coupling *N*-acylpurines with peracylated D-ribose in the presence of Friedel–Crafts catalysts.[3] To apply this method to the synthesis of cytidine, it seemed desirable to synthesize new N^4-acylcytosines (I) which would be soluble in organic solvents.

Cytosine was refluxed in pyridine with isobutyric anhydride or 2-ethylhexanoyl chloride to give N^4-isobutyryl- or -(2-ethylhexanoyl)cytosines (Ia, b), respectively. These compounds, which have branched-chain acyl groups, are fairly soluble in organic solvents. Condensation of Ia or Ib with II in 1,2-dichloroethane (DCE) in the presence of stannic chloride gave N^4-isobutyryl- or -(2-ethylhexanoyl)-2′,3′,5′-tri-*O*-benzoylcytidines (IIIa, b), respectively.

N^4-Octanoyl- and -palmitoylcytosines (Ic, d) described by Colautti *et al.*[4] were almost insoluble in DCE but upon addition of II and stannic chloride, they dissolved and reacted to afford good yields of IIIc and IIId (Table I).

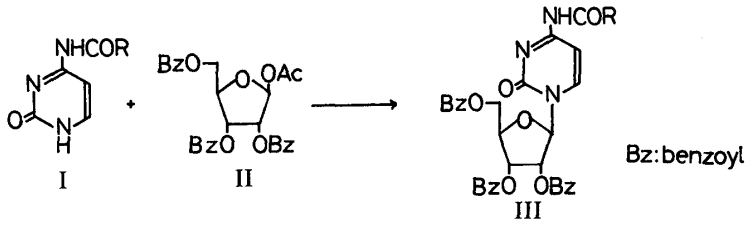
Compound IIIc was deacylated with sodium methoxide in methanol to afford cytidine (overall yield: 65%). Assignment of the structures of III was based on analytical data and ultraviolet (UV) and nuclear magnetic resonance (NMR) spectroscopy.

Transribosylation between I and Acylinosine or Acylguanosine

Purine nucleosides are industrially available sources of D-ribose, and the preparation of II from them has been reported.[5] We studied transfer of a ribosyl moiety from inosine or guanosine to I.

When 2′,3′,5′-tri-*O*-acetyl- or -benzoylinosine[6] (IVa, b) and Ia or Ic were heated in DCE

TABLE I. Condensation of N^4-Acylcytosine (I) and 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (II)

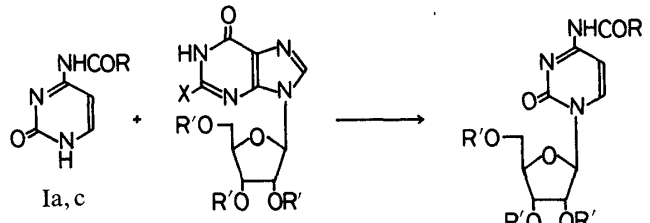


I II III

Run	Reaction conditions	Products R	Yield (%)	mp (°C)	NMR (H_1) (δ, ppm)	Formula[a]
1	r.t. 20 h	iso-C_3H_7 (IIIa)	64	181—183[b]	6.39	$C_{34}H_{31}N_3O_9$
2	r.t. 20 h	$C_4H_9CH(C_2H_5)$ (IIIb)	72	—[c]	6.38	$C_{38}H_{39}N_3O_9$
3	r.t. 20 h	C_7H_{15} (IIIc)	80	—[c]	6.38	$C_{38}H_{39}N_3O_9$
4	Reflux, 4 h	$C_{15}H_{31}$ (IIId)	81	Oil	6.37	$C_{46}H_{55}N_3O_9$[d]

Reaction mixture: Ia—d, 2.4 mmol; II, 2 mmol; $SnCl_4$, 2.4 mmol; DCE, 20 ml. *a*) Elemental analyses were performed for C, H, and N. Unless otherwise stated the results were within ±0.3% of the theoretical values. *b*) Recrystallized from EtOH. *c*) Amorphous powder. The melting point was not determined. *d*) N (Calcd), 5.29; N (Found), 4.91. r.t.: room temperature.

TABLE II. Transribosylation between N^4-Acylcytosines (Ia, c) and Purine Nucleosides (IVa—d)



Ia, c

IVa: X=H, R'=Ac
IVb: X=H, R'=Bz
IVc: X=NH_2, R'=Ac
IVd: X=NHBz, R'=Bz

III

Run	Ribosyl acceptor	Ribosyl donor	Products	R	R'	Yield (%)
1	Ia	IVa	IIIe	iso-C_3H_7	Ac	46
2	Ic	IVa	IIIf	C_7H_{15}	Ac	60
3	Ia	IVb	IIIa	iso-C_3H_7	Bz	30
4	Ic	IVb	IIIc	C_7H_{15}	Bz	59
5	Ic	IVc	IIIf	C_7H_{15}	Ac	26
6	Ic	IVd	IIIc	C_7H_{15}	Bz	18

Reaction mixture: I, 2 mmol; IV, 1 mmol; $SnCl_4$, 1.6 mmol; DCE, 20 ml. Reaction conditions: reflux, 20 h.

in the presence of stannic chloride, the corresponding tetraacylcytidines (IIIa, c, e, f) were afforded in moderate yields, the yields of products from Ic being higher than those from Ia. Compound IIIf was deacylated with sodium hydroxide in aqueous ethanol to afford cytidine

in 74% yield. N^4,O^2-Bistrimethylsilylcytosine and IVb did not react to give 2′,3′,5′-tri-*O*-benzoylcytidine under the same conditions. Heating 2′,3′,5′-tri-*O*-acetyl-[7] or N^2,2′,3′,5′-tetrabenzoylguanosine[8] (IVc, d) with Ic also gave IIIf or IIIc, though in low yields. Thus, acylinosine is a better ribosyl donor than acylguanosine, and also Ic is a better acceptor than Ia (Table II).

Transglycosylation from acylated pyrimidine nucleosides to silylated purines has been reported,[9] and it has been shown that N^4,2′,3′,5′-tetraacetylcytidine is a good ribosyl donor.[10] Transribosylation from acylated purine nucleosides to 2,4-bis(trimethylsilyloxy)-pyrimidine[11] but not to cytosine derivatives has previously been reported. It is of note that transribosylation occurs from acylated purine nucleosides to N^4-acylcytosines but not to N^4,O^2-bis-trimethylsilylcytosine.

There seem to be two reasons why N^4-acylcytosines have not usually been used directly[12] for coupling with sugars: i) since the introduction of the Hilbert–Johnson method, the derivatization of 2-oxopyrimidine to produce an enol ether (V) (Chart 1) has been thought to

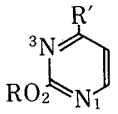


Chart 1

V : R=Me, Me_3Si

be favorable to increase the reactivity of the N^1 atom,[13] and ii) there have been no reports of acylcytosines which are easily soluble in organic solvents except N^4-adamantoylcytosine.[14]

Experimental

Melting points were determined with a micro-melting point apparatus (Yanagimoto) and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian EM-390 spectrometer with tetramethylsilane as an internal standard. UV absorption spectra and infrared (IR) spectra were recorded on a Hitachi EPS-3T recording spectrophotometer and a Hitachi 215 spectrophotometer, respectively.

N^4-Isobutyrylcytosine (Ia)——A mixture of cytosine (2 g) and isobutyric anhydride (6 ml) in pyridine (100 ml) was refluxed for 2 h. The reaction mixture was concentrated *in vacuo* to a small volume, and the resulting precipitate was collected by filtration and recrystallized from MeOH to give Ia as colorless needles (3.2 g, 98%), mp 315—320 °C (dec.). *Anal.* Calcd for $C_8H_{11}N_3O_2$: C, 53.03; H, 6.12; N, 23.19. Found: C, 53.25; H, 6.22; N, 23.19. ^{1}H-NMR (d_6-DMSO) δ: 1.02 (6H, d, Me), 2.23—3.0 (1H, m, CH), 6.86, 7.46 (1H, each d, H_5, H_6).

N^4-(2-Ethylhexanoyl)cytosine (Ib)——A mixture of cytosine (5.55 g) and 2-ethylhexanoyl chloride (10 ml) in pyridine (250 ml) was heated at 70 °C for 1 h. The reaction mixture was evaporated to dryness *in vacuo*, and the residue was washed with MeOH and recrystallized from MeOH to give colorless needles (4 g, 72%), mp 260—263 °C. *Anal.* Calcd for $C_{12}H_{19}N_3O_2$: C, 60.74; H, 8.07; N, 17.71. Found: C, 60.84; H, 8.04; N, 17.83. ^{1}H-NMR ($CDCl_3$) δ: 0.5—2.8 (15H), 7.48, 7.80 (1H, each, d, H_5, H_6).

General Method for the Synthesis of Acylcytidines (III)——Compound I was allowed to react with compound II or IV under the conditions shown in Tables I and II. $CHCl_3$ and 5% aqueous $NaHCO_3$ were added to the reaction mixture and the whole was shaken. The resulting precipitate was removed by filtration through Celite. The aqueous layer was discarded and the $CHCl_3$ layer was evaporated *in vacuo* to dryness. The residue was chromatographed on a column of silica gel (30 g) (eluant: $CHCl_3$ or 1% MeOH in $CHCl_3$). The main fractions were pooled and evaporated to dryness *in vacuo* to give III (Tables I and II).

Isolation of Cytidine——i) $SnCl_4$ (1.5 ml) was added to a suspension of Ic (2.84 g, 7.2 mmol) and II (5 g, 10 mmol) in DCE (150 ml) and the mixture was stirred at room temperature for 20 h. The mixture was washed with water (150 ml) three times. The organic layer was then evaporated *in vacuo* to dryness and the residue was refluxed in 0.5% (w/v) Na–MeOH (100 ml) for 1 h. The mixture was evaporated *in vacuo* to dryness. The residue was dissolved in 0.1 N HCl (100 ml) and the resulting solution was washed with $CHCl_3$. The aqueous layer was loaded on to a column of charcoal (40 g) and the column was washed with water. The column was then eluted with concentrated NH_4OH–50% (0.5 : 100). The eluate was concentrated *in vacuo* to dryness and the residue was recrystallized from 90% EtOH to give cytidine as colorless needles (1.58 g, 65%), mp 210—220 °C. UV $\lambda_{max}^{H_2O}$ nm: 230 (sh), 272. ^{1}H-NMR (D_2O) δ: 5.74 (1H, d, J=4 Hz, H_1·). *Anal.* Calcd for $C_9H_{13}N_3O_5 \cdot H_2O$: C, 41.38; H, 5.79; N, 16.08. Found: C, 41.55; H, 5.62; N, 16.18. The IR spectrum was identical with that of an authentic sample.

ii) A mixture of Ic (4.74 g, 20 mmol), IVa (3.94 g, 10 mmol), and $SnCl_4$ (2 ml) in DCE (200 ml) was refluxed for 6 h. The mixture was washed with water (200 ml × 3) and the organic layer was evaporated *in vacuo* to dryness. The residue was chromatographed on a column of silica gel (200 g) (elution: 1% MeOH–$CHCl_3$) to give IIIf (3 g, 61%). An aliquot was recrystallized from EtOH to give colorless needles, mp 139—140 °C. UV λ_{max}^{EtOH} nm (ε): 252 (11700), 299 (5600). ^{1}H-NMR ($CDCl_3$) δ: 6.06 (1H, d, $H_{1'}$). *Anal.* Calcd for $C_{23}H_{33}N_3O_9$: C, 55.75; H, 6.71; N, 8.48. Found: C, 55.82; H, 6.73; N, 8.41. A 10 N sodium hydroxide solution (6 ml) was added to a solution of IIIf (3 g) in EtOH (60 ml) and the mixture was stirred at room temperature overnight. The mixture was evaporated *in vacuo* to dryness and the residue was dissolved in water. The solution was neutralized with HCl and then treated as described in i) to give colorless needles (1.1 g, 74%), mp 220—222 °C. UV $\lambda_{max}^{H_2O}$ nm: 230 (sh), 273. ^{1}H-NMR (D_2O) δ: 5.80 (1H, d, J=4 Hz, $H_{1'}$).

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