SYNTHESIS OF 5-METHYL-6-AZACYTIDINE*

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Benzoylation of 5-amino-2,3-dihydro-6-methyl-1,2,4-triazin-3-one (*II*) with benzoyl chloride in pyridine affords 5-benzamido-2-benzoyl-2,3-dihydro-6-methyl-1,2,4-triazin-3-one (*III*). Heating of compound *III* in 50% aqueous pyridine or refluxing in methanol results in debenzoylation to 5-benzamido-2,3-dihydro-6-methyl-1,2,4-triazin-3-one (*IV*). Ribosylation of bases *II* and *IV* according to the silylation method affords the protected nucleosides *V* and *VI* which are debenzoylated on treatment with methanolic ammonia under the formation of 5-methyl-6-azacytidine (*I*). Acetylation of the tribenzoyl nucleoside *VI* affords the N-acetyl nucleoside *VII* from which the starting compound *VI* is recovered on deacetylation in boiling methanol.

In connection with syntheses of 6-azaanalogues of pyrimidine nucleosides[1–4] as potential antimetabolites of nucleic acids, 5-methyl-6-azacytidine has been now prepared. A general and relatively easy synthesis of 5-substituted 6-azacytosines[5] including the 6-azaanalogue of the naturally occurring 5-methylcytosine has been developed in this Laboratory some time ago. In the present paper, the ribosylation of 5-methyl-6-azacytosine is described.

Brossmer and Röhm[6] have reported a high yield of about 90% in the ribosylation (mercuri process) of 5-benzyloxymethyl-N^4-benzoylcytosine as a fairly soluble cytosine derivative.

On the other hand, the mercuri process gave a complex mixture of products in the analogous ribosylation of N^4-benzoyl-5-methyl-6-azacytosine (*IV*). Consequently, the silylation method[7] has been now attempted in the preparation of the nucleoside *I*. The starting N-benzoyl derivative *IV* was prepared on benzoylation of 5-methyl-6-azacytosine (*II*) with benzoyl chloride in pyridine. In contrast to paper ref.[6] however, the dibenzoyl derivative *III* was obtained as the primary product in the aza series and the structure of *III* was established by elemental analysis as well as UV, IR, NMR and mass spectra. Most probably, the earlier authors[6] also obtained a dibenzoyl derivative which was converted to the monobenzoyl derivative during the work-up of the reaction mixture. The selective debenzoylation of compound *III*

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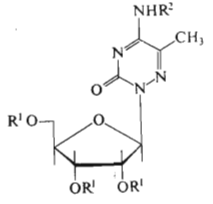
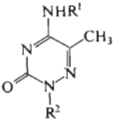
to *IV* may be performed either by heating in 50% aqueous pyridine or refluxing in methanol. The selective N-deacylation in boiling methanol has been the object of some earlier investigations on some cytidine and 6-azacytidine acetyl derivatives[8]; with the 6-azaanalogues, this selective N-deacetylation is by one order of magnitude faster than in the case of the parent cytosine derivatives. The selective methanolysis has been also used in the later debenzoylations of the N-benzoyl derivatives of adenosine[9] and cytosine[6] nucleosides.

By the action of hexamethyldisilazane in the presence of a catalytic amount of trimethylchlorosilane[10], the benzoyl derivative *IV* was converted to the corresponding silylated base which was then condensed with 2,3,5-tri-O-benzoyl-D-rifofuranosyl bromide in acetonitrile in the presence of mercuric acetate to afford the benzoyl derivative *V*. On treatment with methanolic ammonia, compound *V* was converted to the free nucleoside *I* which was in all respects identical with an authentic specimen of 5-methyl-6-azacytidine[11] obtained by the thiation method from 5-methyl-6-azauridine.

In order to elucidate the structure of the tetrabenzoyl nucleoside *V*, methanolysis has been carried out. The expected tribenzoyl nucleoside *VI* was not obtained under the conditions mentioned above but compound *V* underwent a simultaneous N- and O-debenzoylation to afford the free nucleoside *I*. The anomalous behaviour of compound *V* might be explained by the presence of a trace contaminant; this assumption is supported by the course of the methanolysis of the tribenzoyl derivative *VI*. Compound *VI* does not change on refluxing in methanol but after the addition of 20% of the tetrabenzoyl derivative *V*, the mixture of both compounds is smoothly debenzoylated to the free nucleoside *I*.

The nucleoside *I* was also prepared analogously to the above procedure by glycosylation of the free base *II*. All the physical data including the elemental analysis





I; $R^1 = R^2 = H$
V; $R^1 = R^2 = C_6H_5CO$
VI; $R^1 = C_6H_5CO$, $R^2 = H$
VII; $R^1 = C_6H_5CO$, $R^2 = CH_3CO$

II; $R^1 = R^2 = H$
III; $R^1 = R^2 = C_6H_5CO$
IV, $R^1 = C_6H_5CO$, $R^2 = H$

of the resulting tribenzoyl nucleoside *VI* were in accordance with the corresponding formula. By the action of methanolic ammonia, the tribenzoyl derivative *VI* was converted to the free nucleoside *I*, the structure of which was unequivocally confirmed by comparison of physical data with those of the earlier prepared[11] 5-methyl-6-azacytidine and the above prepared specimen.

Since the attempted selective N-debenzoylation of the tetrabenzoyl derivative *V* to the tribenzoyl derivative *VI* failed, some experiments on the reverse benzoylation of compound *VI* to compound *V* were performed, *e.g.*, with benzoyl chloride in pyridine or benzoyl cyanide or benzoic anhydride in the presence of sodium benzoate. None of these benzoylations led to compound *V*. On the other hand, the acetylation of the tribenzoyl nucleoside *VI* gave readily the N-acetyl derivative *VII* from which the starting compound *VI* was recovered by N-deacetylation in boiling methanol.

The UV spectra of 5-methyl-6-azacytidine (*I*) and its acyl derivatives have been measured and interpreted. The UV spectra of the tribenzoyl nucleoside *VI* and its N-acetyl derivative *VII* are almost identical (maxima at 232 and 265 nm). This observation is in accordance with the earlier findings on the similar spectra of 2′,3′,5′-tri-O-acetyl-6-azacytidine and 2′,3′,5′-tri-O-acetyl-N^4-acetyl-6-azacytidine and on the absence of any bathochromic shift which could be due to N-acetylation of 6-azacytidine; on the other hand, the N-acetylation of cytidine and its derivatives is accompanied by a marked bathochromic shift of the long-wavelength band. The UV spectrum of the tetrabenzoyl nucleoside *V* shows however maxima at 231 and 333 nm and a weak maximum at 284 nm. In contrast to N-acetylation, the N-benzoylation thus results in a bathochromic shift of the long-wavelength band. This shift may be also observed in the CD spectrum of the tetrabenzoyl nucleoside *V*. While the CD spectra of derivatives *VI* and *VII* are similar and exhibit a negative maximum at 255 and 256 nm, resp., the CD spectrum of the derivative *V* display negative maxima at 270 and 330 nm. In contrast to 5-methyl-6-azacytidine (*I*), the CD spectra of benzoylated derivatives show an additional significant positive maximum at 235 nm corres-

TABLE I
Thin-Layer Chromatography and Paper Electrophoresis

Compound	R_F Values		Compound	R_F	Mobility, cm	
	S_1	S_2		S_3	E_1	E_2
III	0·91	0·75	*I*	0·16	−5·5	4·3
IV	0·70	0·32				
V	0·73	0·14	*II*	0·34	−5·1	−3·4
VI	0·36	0	6-azacytidine	0·17	−5·5	4·5
VII	0·73	0·17				

ponding to the optically active band of benzoyl groups. The spectrum of 5-methyl--6-azacytidine (*I*) exhibits a positive maximum at 217 nm on which a band of benzoyl groups is partly superimposed in the case of benzoyl derivatives.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block). Analytical samples were dried for 8 h at 25°C/0·01 Torr. Thin-layer chromatography was performed on ready-for-use Silufol UV_{254} silica gel foils (Kavalier Glassworks, Votice, Czechoslovakia) in the solvent systems S_1, benzene–acetone (3 : 2); S_2, benzene–ethyl acetate (4 : 1); and S_3, ethyl acetate–ethanol–acetone–water (5 : 1 : 1 : 1). Electrophoresis was carried out on paper Whatman No 1 at 40 V/cm for 2 h in the buffer solutions E_1, 0·02M-Na_2HPO_4 (pH 7·4), and E_2, borate buffer[12,13] (pH 6·0). The UV spectra were taken on an Optica Milano CF 4 apparatus. The IR spectra were recorded on a Zeiss Model UR 10 spectrophotometer. The CD spectra were measured on a Rousel-Jouan Dichrograph II Model CD 185 spectropolarimeter. The NMR spectra were measured on a Varian HA 100 apparatus at 100 MHz with the use of tetramethylsilane as internal standard. Mass spectra were taken on an AEI MS 902 spectrometer.

5-Benzamido-2-benzoyl-2,3-dihydro-6-methyl-1,2,4-triazin-3-one (*III*)

To a suspension of 5-methyl-6-azacytosine (*II*; 1·26 g; 10 mmol) in pyridine (50 ml) there was added benzoyl chloride (4 ml), the whole shaken for 3 h, and added dropwise under efficient stirring into ice-cold water (150 ml). The precipitate was collected with suction, washed with three 20 ml portions of water and then acetone (5 ml), and crystallised from ethyl acetate to afford 2·15 g (64%) of compound *III*, m.p. 201—203°C. UV spectrum (ethanol): λ_{max} 251 and 303 nm (log ε 4·19 and 4·36, resp.); λ_{min} 267 nm (log ε 4·16). IR spectrum (nujol): sh 1 727 cm^{-1}, 1 715 cm^{-1} (C=O of benzoyls). Mass spectrum: M^+ 334. For $C_{18}H_{14}N_4O_3$ (334·3) calculated: 64·66% C, 4·22% H, 16·76% N; found: 64·55% C, 4·34% H, 16·82% N.

5-Benzamido-2,3-dihydro-6-methyl-1,2,4-triazin-3-one (*IV*)

The dibenzoyl derivative *III* (668 mg; 2 mmol) was dissolved in boiling 50% aqueous pyridine (7 ml) and the solution kept at 0°C for 4 h to deposit crystals. The solid was collected with suction, washed with water (2 ml) and ethanol (2 ml), and crystallised from acetone to afford 120 mg of compound *IV*, m.p. 193—194·5°C. The pyridine filtrate was evaporated, the residue coevaporated with three 5 ml portions of toluene, and crystallised from acetone to afford additional 190 mg of compound *IV*, m.p. 192—194°C. Work-up of mother liquors afforded a crop of 64 mg and the same melting point. Overall yield of compound *IV*, 374 mg (81%). UV spectrum (ethanol): λ_{max} 208 nm and 314 nm (log ε 4·02 and 4·17); λ_{min} 246 nm (log ε 3·76). IR spectrum (nujol): 1 709 cm^{-1} (C=O benzoyl), 3 390 cm^{-1} (NH). Mass spectrum: M^+ 230. For $C_{11}H_{10}N_4O_2$ (230·2) calculated: 57·38% C, 4·38% H, 24·34% N; found: 57·17% C, 4·34% H, 24·20% N.

2′,3′,5′-Tri-O-benzoyl-N^4-benzoyl-5-methyl-6-azacytidine (*V*)

The base *IV* (691 mg; 3 mmol) was coevaporated with 20 ml toluene (10 ml of the distillate) and the residue was treated with hexamethyldisilazane (3 ml) and trimethylchlorosilane (0·3 ml). The whole mixture was refluxed for 2·5 h and evaporated under diminished pressure. The residual silylated base was treated with a solution of 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide (3 mmol) in acetonitrile (20 ml), the molecular sieve Potassit 3 (1 g), and mercuric acetate (480 mg; 1·5 mmol), the whole stirred for 30 min and then kept at room temperature for 12 h. The sieve and other insoluble portions were filtered off and washed with two 5 ml portions of acetonitrile. The filtrate

and washings were combined and evaporated under diminished pressure. The residue was dissolved in chloroform (100 ml), the solution washed with three 25 ml portions of 10% aqueous potassium iodide and two 25 ml portions of water, dried over anhydrous magnesium sulfate, and the chloroform evaporated under diminished pressure. The residue (2·40 g) was chromatographed on a column of silica gel (200 g) in the solvent system benzene–acetone (4 : 1) to afford 641 mg of a chromatographically homogeneous sirup. Crystallisation from di-n-propyl ether afforded 360 mg of compound *V*, m.p. 129—131°C. Work-up of mother liquors gave additional 171 mg of the substance of the same melting point. UV spectrum (ethanol): λ_{max} 231 nm and 333 nm (log ε 4·44 and 4·33, resp.); λ_{min} 257 nm (log ε 3·91). IR spectrum (chlofoform): 3 465 cm^{-1} (NH). CD spectrum (methanol): 218 nm (−12 306), 236·5 nm (+44 536), 270 nm (−7 032), 330 nm (−25 198). NMR spectrum (in deuteriochloroform; chemical shifts in p.p.m.): 2·40 (s, 3 H, 5-CH_3), 4·50—4·90 (m, 3 H, $H_{4'}$, 2 $H_{5'}$), 6·10 (t, 1 H, $H_{3'}$, $J_{H_3'H_4'} = J_{H_2'H_3'} = 5{\cdot}5$ Hz), 6·23 (q, 1 H, $H_{2'}$, $J_{H_1'H_2'} = 2{\cdot}25$ Hz), 6·77 (d, 1 H, $H_{1'}$), 9·90 (broad s, 1 H, NH), 7·20—7·50, 7·80—8·30 (m, 20 H, arom. protons). Mass spectrum: M^+ 661.

2′,3′,5′-Tri-O-benzoyl-5-methyl-6-azacytidine (*VI*)

From a suspension of 5-methyl-6-azacytosine (*II*; 378 mg; 3 mmol) in toluene (18 ml) there was removed by distillation 9 ml of the distillate. Hexamethyldisilazane (2 ml) and trimethylchlorosilane (0·2 ml) was added to the residue, the whole refluxed for 8 h, and evaporated under diminished pressure. 2,3,5-Tri-O-benzoyl-D-ribofuranosyl bromide (3 mmol) in acetonitrile (12 ml) was added along with molecular sieve Potassit 3 (1 g) to the residue, the mixture stirred for 2 min, and then treated with mercuric acetate (0·25 g). The whole mixture was stirred at room temperature for 4 h and evaporated under diminished pressure. The residue was dissolved in chloroform (100 ml), the solution washed with three 25 ml portions of 10% aqueous potassium iodide and two 25 ml portions of water, dried over anhydrous magnesium sulfate, and evaporated under diminished pressure. The residue was chromatographed on a column of silica gel (200 g) in the solvent system benzene–acetone (1 : 1) to afford 1·05 g of a chromatographically homogeneous solid. Crystallisation from ethanol yielded 826 mg of compound *VI*, m.p. 180—183°C; work-up of mother liquors afforded additional 125 mg of the same substance. Optical rotation: $[\alpha]_D^{25}$ −92·8° (*c* 0·47; ethyl acetate). UV spectrum (ethanol): λ_{max} 232 nm and 266 nm (log ε 4·60 and 4·03, resp.); λ_{min} 256 nm (log ε 4·01). CD spectrum (methanol): 215 nm (+2 401), 221 nm (−3 362), 233 nm (+29 780). 255 nm (−24 490). NMR spectrum (in deuteriochloroform; chemical shifts in p.p.m.): 2·17 (s, 3 H, 5-CH_3), 6·03 (t, 1 H, $H_{3'}$, $J_{H_3'H_4'} = J_{H_2'H_3'} = 5{\cdot}5$ Hz), 6·16 (q, 1 H, $H_{2'}$, $J_{H_1'H_2'} = 2{\cdot}25$ Hz), 6·64 (d, 1 H, H_1'), 7·20—7·60, 7·85—8·10 (m, 15 H, arom. protons). For $C_{30}H_{26}N_4O_8$ (570·5) calculated: 63·15% C, 4·59% H, 9·82% N; found: 62·86% C, 4·78% H, 9·69% N.

N^4-Acetyl-2′,3′,5′-tri-O-benzoyl-5-methyl-6-azacytidine (*VII*)

A mixture of the tribenzoyl derivative *VI* (143 mg; 0·25 mmol), pyridine (1 ml), and acetic anhydride (0·5 ml) was kept at room temperature for 24 h and evaporated under diminished pressure. The residue was coevaporated with three 5 ml portions of toluene and finally chromatographed on a column of silica gel in the solvent system benzene–acetone (3 : 1) to afford 118 mg of compound *VII* in the form of a chromatographically homogeneous solid foam. Optical rotation: $[\alpha]_D^{25}$ −87·9° (*c* 0·48; ethyl acetate). UV spectrum (ethanol): λ_{max} 232 nm and 265 nm (log ε 4·37 and 4·02, resp.); λ_{min} 254 nm (log ε 3·99). CD spectrum (methanol): 234 nm (+32 210), 256 nm (−19 440). For $C_{32}H_{28}N_4O_9$ (612·6) calculated: 62·74% C, 4·61% H, 9·15% N; found: 62·64% C, 4·55% H, 8·92% N.

Deacetylation. A solution of the nucleoside derivative *VII* (61 mg; 0·1 mmol) in methanol (5 ml) was refluxed and the course of methanolysis checked by thin-layer chromatography in the solvent systems 3 : 1 and 2 : 3 benzene–acetone. When the reaction was complete (90 min), the methanol was evaporated under diminished pressure and the residue crystallised from ethanol Yield, 43 mg of a substance identical in all respects with the tribenzoyl derivative *VI*.

5-Methyl-6-azacytidine (*I*)

A. A solution of compound *V* (330 mg; 0·5 mmol) in 18% methanolic ammonia was kept at room temperature for 3 days and evaporated under diminished pressure. The residue was crystallised from a mixture (1 : 1) of ethanol and methanol to afford 95 mg of compound *I*, m.p. 238 to 239°C, undepressed on admixture with an authentic specimen[11]. CD spectrum (water): 217 nm (+4 560), 240·5 nm (−3 610), 279 nm (−7 037). For $C_9H_{14}N_4O_5$ (258·2) calculated: 41·86% C, 5·46% H, 21·70% N; found: 42·04% C, 5·48% H, 21·80% N.

B. The tribenzoyl derivative *VI* (286 mg; 0·5 mmol) afforded by a similar process 104 mg of a substance identical with authentic 5-methyl-6-azacytidine[11] (*I*).

C. A solution of compound *V* (50 mg) in methanol (10 ml) was refluxed for 4 h, allowed to cool, and evaporated under diminished pressure. The residue was crystallised from a mixture (1 : 1) of ethanol and methanol to afford 14·5 mg of a substance identical with that obtained by procedure *A*.

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