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Aminonucleosides and their derivatives. IV[I] Synthesis of the 3'-amino-3'-deoxynucleoside 5'-phosphates

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ABSTRACT

A new procedure has been developed for the synthesis of 3'-amino-3'-deoxyribonucleosides of adenine, cytosine and uracil by condensing the trimethylsilylated bases with peracylated 3-azido-3-deoxyribose derivative. The azido group could subsequently be reduced to amino. The 5'-phosphates of these nucleosides have been prepared and the analogues have been tested for their ability to stimulate the ribosome-catalyzed reaction of 3'(2')-O-(N-formylmethionyl)adenosine 5'-phosphate with phenylalanyl-tRNA.

INTRODUCTION

Nucleosides, in which one of the hydroxyl groups of the sugar residue is substituted by the amino function, are of interest to present-day molecular biologists and enzymologists.

Introduction of the 3'-amino-3'-deoxy- and the 2'-amino-2'-deoxyadenosine into the tRNA 3'-terminus was among the methods which enabled the direction of aminoacylation of different tRNA types to be determined [2]. The 5'-triphosphates of the 2'- and the 3'-aminonucleosides appeared to be effective inhibitors of the DNA-dependent RNA-polymerase from E.coli [3]. Various 3'-amino-3'-deoxynucleosides may also be used as a tool for the study of the peptidyltransferase center of ribosomes.

The amino acid derivatives of the 3'-amino-3'-deoxyadenosine bound to the acceptor site of the peptidyltransferase center are known to act as inhibitors of protein synthesis [4]. The results obtained indicated that the acyl-amino acid derivatives of 3'-amino-3'-deoxyadenosine 5'-phosphate bind to

the donor site of the ribosomal peptidyltransferase and thus inhibit the transfer of the acylamino acid residue from CACCA-(AcLeu) to the phenylalanyl-tRNA [5].

With these facts in mind it was felt to be important to develop a procedure which would enable substantial amounts of the 3'-amino-3'-deoxynucleosides and their 5'-phosphates to be prepared.

RESULTS AND DISCUSSION

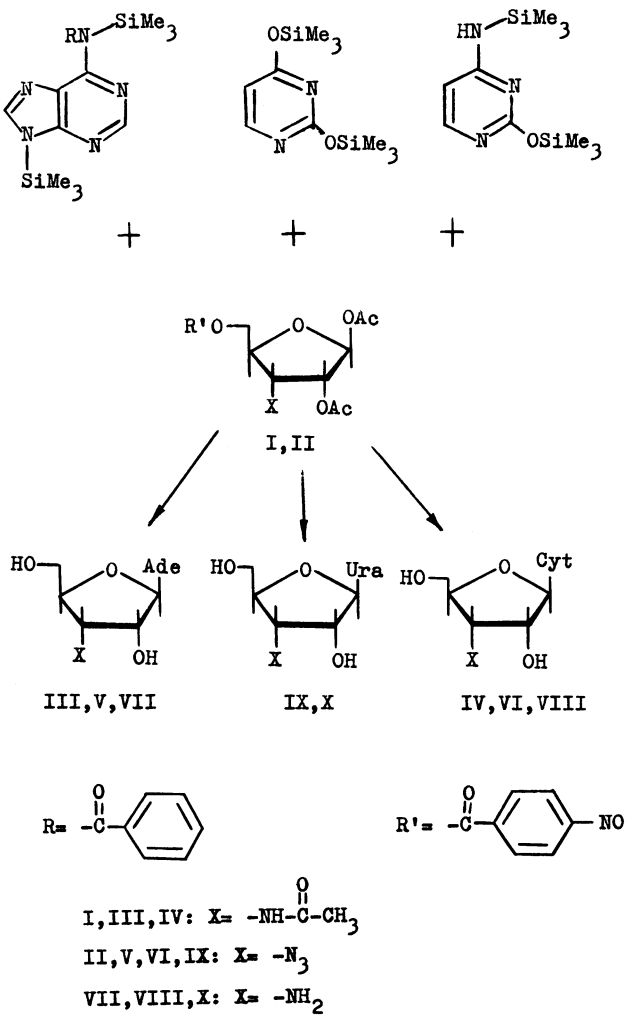
Earlier we have reported the synthesis of the 3'-amino--3'-deoxyuridine [6] obtained by stepwise modification of uridine and also the method for the preparation of 3'-amino-3'--deoxyadenosine [7]. In this paper we wish to report the synthesis of the adenine, cytosine and uracil containing 3'--amino-3'-deoxynucleosides and their 5'-phosphates. The aminonucleosides were obtained by means of glycosidation of the silylated N-benzoyladenine, cytosine and uracil[9] either with the earlier reported 3-acetamido-3-deoxy-1,2-di-O-acetyl-5-O-p-nitrobenzoyl-ß-D-ribofuranose [8] and the subsequent removal of the protective groups, or with the 3-azido-3-deoxy-1,2-di-O-acetyl-5-O-p-nitrobenzoyl-ß-D-ribofuranose and the subsequent reduction of the azido group (Scheme 1).

Synthesis of VII via azidonucleoside has been achieved by us before. However, we would like to describe here a simplified procedure for obtaining VII, and also the synthesis of VIII and X via appropriate azidonucleosides.

The starting material for the synthesis of II was 1,2--O-isopropylidene-3-O-p-toluenesulfonyl-ß-D-xylofuranose (XV) prepared from 1,2-O-isopropylidene-α-D-xylofuranose (XI). 1,2:5,6-Di-O-isopropylidene-3-O-p-toluenesulfonyl-α--D-glucofuranose (XIII) can also serve as a starting material for the synthesis of XV [7]. The further synthesis of II was performed as described in [7] with a few modifications (Scheme 2).

The glycosidation of the silylated N^6-benzoyladenine and cytosine with I was performed in the 1,2-dichloroethane-acetonitrile mixture in the presence of stannic chloride. Acetamidonucleosides III and IV were obtained after the gly-

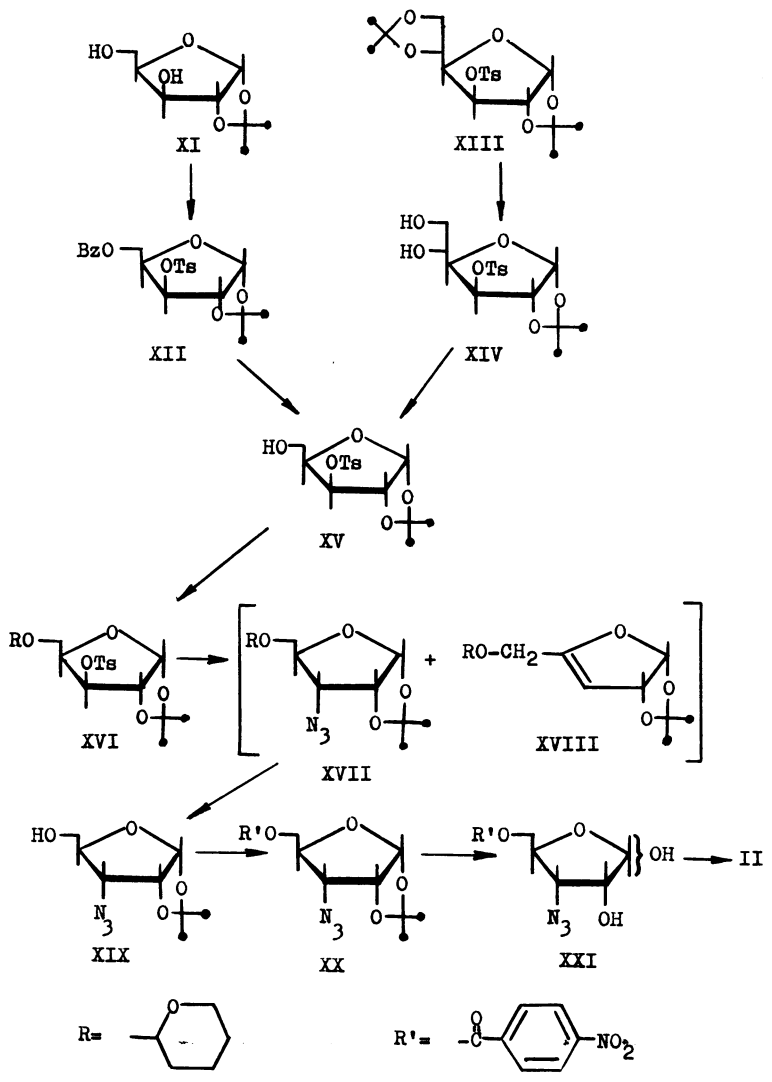
Scheme I



cosidation procedure and the removal of the acyl groups with methanolic ammonia in a good yield. Removal of the acetyl group from the aliphatic amino function in III and IV was observed to proceed under drastic conditions (MeONa/MeOH, Δ, 24 hr). The yield at that step did not exceed 50% and considerable darkening of the reaction mixture made the isolation of the desired aminonucleosides difficult.

Thy glycosidation of silylated N^6-benzoyladenine and

Scheme 2



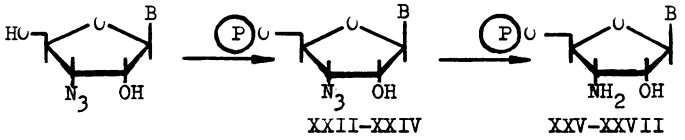
cytosine with II in 1,2-dichloroethane in the presence of stannic chloride gave (after the removal of acyl groups) azides V and VI with an overall yield of 68% and 72.5% from II, respectively. The reaction of silylated uracil with II in 1,2-dichloroethane in the presence of stannic chloride

gave the mixture of the desired N_1-riboside and N_1,N_3-bisriboside, as was obvious from the PMR spectra of the separated products. The formation of bisribosides in the reaction of silylated uracyls with peracylated sugars in 1,2-dichloroethane in the presence of stannic chloride was reported by Vorbrüggen et al. [10]. When the reaction was run in acetonitrile, the overall yield of azide IX from II was 97% (no N_3-riboside or N_1,N_3-bisriboside was detected). The reduction of the azido group in nucleosides V, VI and IX with triphenylphosphine in pyridine, followed by treatment of the reaction mixture with ammonium hydroxide, resulted in amines VII, VIII and X in a high yield.

The simplicity and high yield of the aminonucleoside via the azido nucleoside procedure in comparison to the procedure via the acetamidonucleoside as well as the advantages of synthesis of II (against I), offer the possibility of obtaining larger quantities of sugar in a shorter space of time and suggests that the route via the azide is more promising. Another advantage of the azido group is that it may act as a completely inert form of the blocked amino function and can be easily transformed to the amino group in the nucleotide or oligonucleotide structures [1].

Hereafter we shall report the procedure for the synthesis of 3'-amino-3'-deoxynucleoside 5'-phosphates starting from azides V, VI and IX (Scheme 3). Phosphorylation of V, VI and IX was achieved with phosphorus oxychloride in triethylphosphate, following a modified procedure [11]. Reduction of the azidonucleotides XXII-XXIV with triphenylphosphine in pyridine in the presence of ammonium hydroxide resulted in

Scheme 3



XXII : B = Ade; XXV : B = Ade;
XXIII : B = Cyt; XXVI: B = Cyt;
XXIV : B = Ura; XXVII: B = Ura.

the production of aminonucleotides XXV-XXVII in high yield. The spectral, chromatographic and electrophoretic characteristics of the obtained nucleosides and nucleotides are given in Table I.

Table 2 illustrates the PMR spectra of the obtained nucleosides and nucleotides. The spectra of V and VII are similar to those reported in [12]. The value $J_{1',2'}$ decreases in the sequence azide-amine-acetamide. This may indicate the shift of the conformational N⇌S equilibrium of the ribofuranose cycle to the N-region in this sequence [13].

As it was reported earlier the cytidine and its 5'-phosphate promote the ribosome catalyzed reaction of the 3'-terminal fragment of the peptidyl-tRNA-3'(2')-O-(N-formylmethionyl)adenosine 5'-phosphate with the phenylalanyl-tRNA [14,15].

TABLE 1

SPECTRAL*, CHROMATOGRAPHIC AND ELECTROPHORETIC CHARACTERISTICS OF THE OBTAINED NUCLEOSIDES AND NUCLEOTIDES

Compound	$\lambda_{max}^{pH\ 7.0}$ nm(ε)	R_f in system A	R_f in system B	$E_{Ado}^{pH\ 2.5}$	$E_{pC}^{pH\ 7.5}$
III	259(15300)	0.52	0.85	1.06	-
V	259(15200)	0.55	0.94	0.91	-
VII	259(15100)	0.33	0.71	1.85	-
XXII	259(15570)	0.36	0.31	0.00	0.86
XXV	259(15740)	0.20	0.18	0.88	0.80
IV	270(8690)	0.29	0.68	1.36	-
VI	270(8750)	0.44	0.83	1.00	-
VIII	270(8960)	0.21	0.56	1.95	-
XXIII	270(8700)	0.35	0.20	0.00	1.00
XXVI	270(8620)	0.15	0.09	1.05	0.91
IX	261(9830)	0.63	0.66	0.00	-
X	261(9420)	0.35	0.46	1.33	-
XXIV	261(9930)	0.38	0.16	0.00	1.08
XXVII	261(9610)	0.25	0.06	0.14	1.00

*UV-spectra were recorded in 0.1 M Na-phosphate buffer (pH 7.0).

TABLE 2

CHEMICAL SHIFTS (ppm) AND SPIN-SPIN COUPLING CONSTANTS (JHz) OF THE OBTAINED NUCLEOSIDES AND NUCLEOTIDES*

Type of proton	Compound					
	III**	V**	VII**	VII***	XXII***	XXV***
H-8	8.33 s	8.47 s	8.47 s	8.38 s	8.58 s	8.38 s
H-2	8.08 s	8.27 s	8.25 s	8.28 s	8.10 s	7.97 s
H-1'($J_{1',2'}$)	5.90d(2.0)	6.01d(6.0)	6.03d(3.0)	6.16d(3.0)	6.03d(5.8)	5.93d(2.8)
H-2'($J_{2',3'}$)		5.11dd(5.5)	4.40dd(5.5)		5.01dd(5.0)	4.48dd(5.5)
H-3'($J_{3',4'}$)	+ HOD:	4.41dd(5.5)		+ HOD:	+ HOD	3.14dd(7.3)
H-4'($J_{4',5'a,b}$)	4.52-3.15 m	4.07dd(3.5)	+ HOD:	5.07-3.87 m	4.63-4.33 m	3.06-3.83
H-5'a,b($J_{4',5'a,b}$)		3.73t(3.5)	4.00-3.20 m		4.15 u	
CH_3CO	2.00 s	-	-	-	-	-

Type of proton	IV***	VI**	VIII***	XXIII***	XXVI***
H-6 ($J_{6,5}$)	7.97d(7.5)	7.92d(8.0)	7.88d(7.5)	8.03d(7.5)	7.93d(7.5)
H-5 ($J_{5,6}$)	6.06d(7.5)	5.87d(8.0)	5.93d(7.5)	6.10d(7.5)	6.07d(7.5)
H-1'($J_{1',2'}$)	5.87d(1.0)	5.85d(4.0)	5.81d(1.5)	5.95d(4.0)	5.90d)2.0)
H-2'($J_{2',3'}$)		4.45t(4.0)	4.20dd(5.5)	4.60t(4.0)	4.72-4.33 m H-2' + H-4'
H-3'($J_{3',4'}$)	+ HOD: 4.95-3.70 m	+ HOD: 4.12-3.91 m	3.30dd(8.0)	+ HOD: 4.43-4.08 m	3.95dd($J_{3',2'}$ 5.5; $J_{3',4'}$ 8.0)
H-4'($J_{4',5'a,b}$)			4.15-3.72 m		4.72-4.33 m H-2'+ H-4'

Type of proton	IV***	VI**	VIII***	XXIII***	XXVI***
H-5'($J_{4',5'a,b}$)		3.70t(4.0)			4.18 u
CH_3CO	2.08	-	-	-	-

Type of proton	IX***	X***	XXIV***	XXVII***
H-6($J_{6,5}$)	7.93d(8.0)	7.99d(8.0)	8.05d(8.0)	8.02d(8.0)
H-5($J_{5,6}$)	5.95d(8.0)	5.93d(8.0)	6.02d(8.0)	5.94d(8.0)
H-1'($J_{1',2'}$)	5.93d(4.0)	5.89d(1.0)	5.99d(5.5)	5.93d(2.5)
H-2'($J_{2',3'}$)	4.67dd(5.0)	4.37dd(5.5)	4.68t(5.5)	H-2' + H-4' + HOD: 5.10-4.30m
H-3'($J_{3',4'}$)	+ HOD:	3.46dd(9.5)	+ HOD:	3.94t($J_{3',2'}$= $J_{3',4'}$= 7.0)
H-4'($J_{4',5a,b}$)	4.33-4.11 m	+ HOD:	4.44-4.26 m	H-4' + H-2' + HOD: 5.10-4.30m
H-5'a,b($J_{4',5'a,b}$)	3.93dd($J_{4',5'a}$ 3.0; $J_{4',5'b}$ 4.0)	4.19-3.71 m	4.10 u	4.10d(4.0)

*Prior to recording the spectra all the compounds were twice co-evaporated with D_2O;

** Solution in DMSO-d_6;

*** Solution in D_2O.

s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet, u = unresolved.

The analogs of cytidine so obtained were tested for their stimulating ability. Fig. I demonstrates that VIII and 2'-amino-2'-deoxycytidine (a kind gift of prof. F.Eckstein) stimulate the ribosomal process in practically equal measure and 2-3 times as actively as cytidine. We can give no expla-

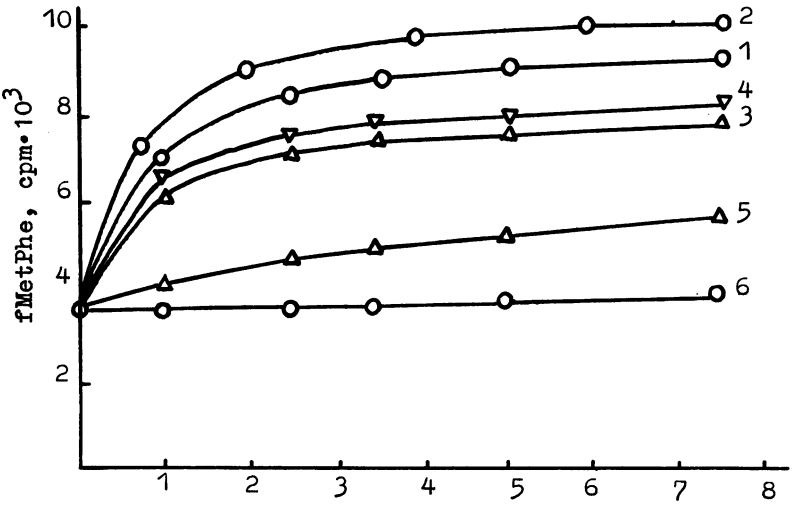


Fig.1. Stimulation of the reaction of 3'(2')-O-(N-formylmethionyl)adenosine 5'-phosphate with [^{3}H]Phe-tRNA using: I - cytidine 5'-phosphate; 2 - XXVI; 3 - VIII; 4 - 2'-amino-2'-deoxycytidine; 5 -cytidine; 6 - XXIII. The reaction mixture contained ribosomes 110 pmol, [^{3}H]Phe-tRNA (3-4).10^4 cpm (0.7-0.9 pmol); incubation and further treatment was carried out according to [15].

nation for this difference so far. At the same time XXVI is even more effective than cytidine 5'-phosphate - the most active analog, reported in [14], whereas the azide XXIII does not influence the reaction.

EXPERIMENTAL

Compound XI was obtained as described in [16] and I synthesized according to [8]. Thin layer chromatography was run on the "Silufol UV254" plates (Kavalier, Czechoslovakia) in the n-butanol-water-acetic acid (5:3:2, system A), the isopropanol-NH_4OH-water (7:1:2, system B), the ethylacetate-benzene (3:17, system C), the ethylacetate-benzene (1:4, system D). The paper electrophoresis was carried out on "Filtrak №3" (GDR) paper in a 6% acetic acid, pH 2.5, or in 0.025 M triet-

hylammonium hydrogen carbonate buffer, pH 7.5. UV spectra were taken with "Beckman 25" (USA), IR spectra with "UR-10" (GDR). PMR spectra were registered with "Varian XL-100" (USA) in $CDCl_3$ with tetramethylsilane as the internal standard or in DMSO or D_2O with tert-butanol as the internal standard (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, dt = doublet of triplets, m = multiplet, u = unresolved; in ppm scale).

1,2-O-Isopropylidene-3-O-p-toluenesulfonyl-5-O-benzoyl-α-D-xylofuranose (XII)

To a cooled solution (0°) of XI (81 g) in dry pyridine (120 ml) benzoyl chloride (50 ml) in dry pyridine (70 ml) was added dropwise and the mixture was kept overnight at 4°. p-Toluenesulfonyl chloride (165 g) was then added and the mixture was kept for 4 hr at room temperature. The mixture was poured onto 350 ml of crushed ice, extracted with chloroform (3 x 200 ml). The extracts were combined, washed with saturated sodium hydrogen carbonate, dried over Na_2SO_4, evaporated to dryness and co-evaporated with toluene (2 x 100 ml)

The residue was dissolved in 50 ml of benzene and filtered through the silica gel column (3 x 20 cm) and the solid was washed with benzene. The filtrate was evaporated to dryness and the residue was crystallized from ethanol. Yield 142 g (73.5%) of XII; m.p. 90-92°; R_f 0.58 (chloroform); PMR in $CDCl_3$: 7.98-7.01 (m, 11 H, $H_3(C_6\underline{H}_4SO_2 + C_6\underline{H}_5CO)$, 5.96 (d, 1H, $J_{1,2}$ 4HZ, H-1), 4.93d, 1H, $J_{2,1}$ 4Hz, $J_{2,3}$ 0Hz, H-2), 4.66--4.19 (m, 3H, H-4 + H-5a,b), 2.26(s, 3H, $\underline{H}_3C-C_6H_4SO_2-$), 1.49 and 1.30(2s, 6H, $2CH_3$). Anal. Calcd. for $C_{22}H_{24}O_8S$(448.48): :58.92%C, 5.39%H; Found: 58.83%C, 5.43%H.

1,2-O-Isopropylidene-3-O-p-toluenesulfonyl-α-D-xylofuranose (XV)

To a solution of XII (72 g) in ethanol KOH (14 g) in water (20 ml) was added. The mixture was boiled for 20 min, cooled to a room temperature, neutralized with 4% HCl (pH 7) and evaporated to dryness. Chloroform (250 ml) and water

(250 ml) were then added to the residue. The organic layer was separated, and the water layer extracted with chloroform (2 x 100 ml). The organic extracts were combined, washed with 10% sodium hydrogen carbonate solution, dried over Na_2SO_4 and evaporated to dryness. Crystallization of the residue from hexane-chloroform gave 42 g (76%) of XV; m.p. 83-84°, R_f 0.24 in C; PMR in $CDCl_3$: 7.74 and 7.30 (2d, 4H, J8Hz, H_3C $C_6\underline{H}_4SO_2$-), 5.84(d, 1H, $J_{1,2}$4Hz, H-1), 4.84(d, 1H, $J_{3,2}$0Hz, $J_{3,4}$3Hz, H-3), 4.53 (d, 1H, $J_{2,1}$4Hz, $J_{2,3}$0Hz, H-2), 4.29 (dt, 1H, $J_{4,3}$ 3Hz, $J_{4,5,a,b}$ 8Hz, H-4), 3.65 (u, 2H, H-5a,b), 2.40(s, 3H, $\underline{H}_3C$-$C_6H_4SO_2$-), 1.41 and 1.22(2S, 6H, $2CH_3$). Anal calcd. for $C_{15}H_{20}O_7S$(344.38):52.32%C, 5.85%H; Found: 52.28%C, 5.89%H.

1,2-O-Isopropylidene-3-O-p-toluenesulfonyl-5-O-tetrahydropyranyl-α-D-xylofuranose (XVI)

To a solution of XV (25 g) in dry N,N-dimethylformamide (80 ml), containing 2,3-dihydropyrane (20 ml), a 6% solution of HCl in N,N-dimethylformamide (8 ml) was added. After 20 min triethylamine (10 ml) was added to a stirred solution, the mixture was poured into water (200 ml) and extracted with ether (3 x 100 ml). The extracts were combined, washed with water (2 x 100 ml), dried over Na_2SO_4 and evaporated. The residue was co-evaporated with pyridine (75 ml) then with toluene (3 x 75 ml) and finally dried in vacuo to give an oily residue. Yield 31 g (99%) of diasteriomers XVI, R_f 0.55 in C.

3-Azido-3-deoxy-1,2-O-isopropylidene-α-D-ribofuranose (XIX)

A solution of XVI (29 g) in hexamethylphosphoric triamide (150 ml), containing lithium azide (18 g), was stirred for 3 hrs at 150°. The mixture was cooled to room temperature, diluted with water (300 ml) and extracted with ether (6 x 200 ml). The combined extracts were washed with water (3 x 150 ml) and evaporated. The residue was co-evaporated with toluene (3 x 50 ml), dissolved in chloroform (50 ml) and filtered through a silica gel column (3 x 10 cm). The silica gel was

washed with chloroform. The filtrate and washings were combined and evaporated to dryness (TLC in D: R_f 0.54 and R_f 0.45, presumably XVIII and XVII).

A 75% aqueous acetic acid (300 ml) was then added to the residue and solution was kept for 15 hrs at room temperature. The mixture was evaporated, co-evaporated with n-butanol (2 x 50 ml) and then with toluene. The solution of the residue in system C (10 ml) was applied to the silica gel column (3 x 30 cm). The elution was run, using the same system. The fractions, containing pure azide XIX (control-TLC in D), were combined, evaporated and the residue was dried in vacuo. Yield 7.9 g (54%) of XIX, R_f 0.20 in D; IR: ν_{max} $CHCl_3$ 2 114 cm^{-1} ($-N_3$); PMR in $CDCl_3$: 5.76(d, 1H, $J_{1,2}$ 4Hz, H-1), 4.70 (t, 1H, $J_{2,1}$ 4Hz, $J_{2,3}$ 4 Hz, H-2), 4.20-3.40(m, 4H, H-3 + + H-4 + H-5a,b), 1.52 and 1.32 (2s, 6H, $2CH_3$).

3-Azido-3-deoxy-1,2-O-isopropylidene-5-O-p-nitrobenzoyl-α-D-ribofuranose (XX)

To a solution of XIX (7.5 g) in dry pyridine (70 ml) and 1,2-dichloroethane (30 ml), cooled to 0°, p-nitrobenzoylchloride (7.1 g) was added, the mixture was kept for 1 hr at room temperature and poured onto 300 ml of crushed ice. The reaction mixture was extracted with chloroform (2 x 200 ml), the combined extracts were thoroughly washed with the saturated $NaHCO_3$, dried over Na_2SO_4, evaporated and then twice co-evaporated with toluene. The syrupy residue was filtered through the silica gel column (3 x 20 cm), the silica gel was washed with chloroform. The filtrate and washing were combined and evaporated. The residue was crystallized from ethanol. Yield 9.1 g (71%) of XVIII (needles), m.p. 81-83°, R_f 0.68 in C, IR: ν_{max} $CHCl_3$ 2114 cm^{-1} ($-N_3$), PMR in $CDCl_3$: : 8.20(d, 4H, J1, 5 Hz, O_2N C_6H_4CO-, 5.81(d, 1H, $J_{1,2}$ 4Hz, H-1), 4.75(t, 1H, $J_{2,1}$ 4Hz, $J_{2,3}$ 4Hz, H-2), 4.67-4.25(m, 3H, H-4+H-5a,b), 3.32(dd, 1H, $J_{3,2}$ 4Hz, $J_{3,4}$ 0Hz, H-3), 1.55 and 1.33 (2C, 6H, $2CH_3$). Anal. Calcd. for $C_{15}H_{16}N_4O_7$(364.3): :49.45%C, 4.43%H, 15.38% N; Found: 49.21%C, 4.52% H, 15.18%N.

3-Azido-3-deoxy-5-O-p-nitrobenzoyl-D-ribofuranoses (XXI)

A suspension of XVIII (9 g) was stirred in a 70% formic

acid (300 ml) for 1.5 hr at 50°. The reaction mixture was evaporated and then co-evaporated with n-butanol and toluene. The residue was dried in vacuo. Yield 7.8 g (96%) of XIX, R_f 0.18 in D.

3-Azido-3-deoxy-1,2-di-O-acetyl-5-O-p-nitrobenzoyl--ß-D-ribofuranose (II)

Acetic anhydride (90 ml) was added to a solution of XXI (7.5 g) in dry pyridine (120 ml). The reaction mixture was kept for 2 hrs at room temperature, poured onto 300 ml of crushed ice and extracted with chloroform (3 x 150 ml). The extracts were carefully washed with the saturated aqueous $NaHCO_3$, dried over Na_2SO_4, evaporated and the residue was twice co-evaporated with toluene. Crystallization of the residue from chloroform-hexane yielded 8.7 g (89%) of II (needles); m.p. 103-104°, R_f 0.67 in D, IR: ν_{max} $CHCl_3$ 2119 cm^{-1} ($-N_3$), PMR in $CDCl_3$: 8.22(s, 4H, $O_2N\underline{C_6H_4}CO-$), 6.12(s, 1H, $J_{1,2}$ 0Hz, H-1), 5.35(d, 1H, $J_{2,1}$ 0Hz, $J_{2,3}$ 4.5Hz, H-2), 4.49 (U, 2H, H-$5_{a,b}$), 4.35(dt, 1H, $J_{4,3}$ 8Hz, $J_{4,5a,b}$ 5Hz, H-4), 4.10 (dd, 1H, $J_{3,2}$ 4.5 Hz, $J_{3,4}$ 8Hz, H-3), 2.12 and 1.91(2s, 6H, 2 $\underline{CH_3}CO-$). Anal. Calcd. for $C_{16}H_{16}N_4O_9$ (408.3): 47.06%C, 3.95%H, 13.72%N; Found: 46.90%C, 3.98%H, 13.57%N.

3'-Acetamido-3'-deoxyadenosine (III)

A solution of N^6-benzoyl-N^6, 9-bis(trimethylsilyl)adenine (prepared of 3.65 g of N^6-benzoyladenine) in dry 1,2-dichloroethane (20 ml) was added to a solution of I (65 g) in a mixture of dry 1,2-dichloroethane (60 ml) and dry acetonitrile (20 ml). A solution of stannic chloride (2 ml) in dry 1,2-dichloroethane was then added to the mixture. The reaction mixture was heated at reflux for 8 hrs, cooled, diluted with 50 ml of chloroform, neutralized with saturated sodium hydrogen carbonate (100 ml) and filtered through a layer of Supercel Hyflo. The organic layer was separated, washed with saturated sodium hydrogen carbonate, dried over Na_2SO_4 and evaporated to dryness. The saturated at 0° solution of ammonia in methanol (75 ml) was added to the residue and the mixture was

kept for 30 hrs at room temperature. The solution was evaporated to dryness and the residue was washed with ether (5 x 25 ml). The washings were combined, extracted with 50 ml of water. The aqueous phase was separated, combined with the precipitate and evaporated to dryness. The residue was crystallized from ethanol. Yield 2.2 g. The mother liquor was evaporated, the residue was dissolved in water and applied to the Dowex-50 (H^+) column (3 x 3 cm). The resin was washed with a 30% ethanol (250 ml) and III was eluted with a 3% aqueous ammonia. The eluate was evaporated to dryness. Crystallization of the residue from ethanol afforded additional 0.8 g of II. The total yield of III was 3 g (63.7%); m.p. 247-249° dec (lit. [17] 249° dec).

3'-Acetamido-3'-deoxycytidine (IV)

A solution of N^4, 2-bis(trimethylsilyl)cytosine (prepared of 0.99 g cytosine) in dry 1,2-dichloroethane (20 ml) was added to a solution of I (4.26 g) in a mixture of dry 1,2-dichloroethane (40 ml) and acetonitrile (20 ml). A solution of stannic chloride (1.5 ml) in dry dichloroethane (10 ml) was then added to the mixture. The reaction mixture was heated at reflux for 1.5 hr, cooled, diluted with 30 ml of chloroform, neutralized with saturated sodium hydrogen carbonate (75 ml), filtered through a layer of Super-cel Hyflo and evaporated to dryness. A 10% aqueous ammonia (100 ml) was then added to a residue and the mixture was kept overnight at room temperature. The solution was evaporated to dryness, the residue dissolved in 75 ml of water and applied to the Dowex-50 (H^+) column (5 x 15 cm). The resin was washed with 0.5 l of water and the substance was eluted with a 3% ammonium hydroxide. The solution was evaporated to dryness, the residue dissolved in the minimal amount of water and applied to the Dowex 1 x 4 (OH^-) column (4 x 10 cm) and eluted with water. The fractions, containing the substance with R_f 0.52 in A were collected and freese-dried. The yield of IV was 1.71 g (60%).

3'-Azido-3'-deoxyadenosine (V)

To a solution of II (5.4 g) and N^6-benzoyl-N^6, 9-bis(trimethylsilyl)adenine (5.4 g) in dry 1,2-dichloroethane (100 ml) a solution of stannic chloride (2 ml) in dry 1,2-dichloroethane (10 ml) was added and the mixture was heated at reflux for 2 hrs. The mixture was then cooled, diluted with chloroform (100 ml), neutralized with saturated sodium hydrogen carbonate solution (150 ml) and filtered through a layer of Super-cel Hyflo. The organic layer was separated, dried over Na_2SO_4 and evaporated to dryness. The saturated at 0° solution of ammonia in methanol (75 ml) was then added to the residue, the mixture was kept for 3 hrs at room temperature and evaporated to dryness. The residue was washed with ether (3x30 ml). The washings were combined, extracted with water (100 ml). The aqueous layer was separated, combined with the precipitate and evaporated. The residue was dissolved in a minimal amount of hot water and applied to the Dowex-50 (H^+) column (3 x 10 cm). The resin was thoroughly washed with a 30% aqueous ethanol and the substance was eluted with the 25% NH_4OH-ethanol-water (1:2:7). The solvents were evaporated and the residue was crystallized from water. The yield of V was 2.65 g (68%). M.p. 219-220° (lit [12] 218-220°). Anal. Calcd. for $C_{10}H_{12}O_8N_3$ (292.2): 41.10% C, 4.14%H, 38.34%N; found: 41.00%C, 4.17%H, 38.21%N.

3'-Azido-3'-deoxycytidine (VI)

To a solution of N^4, 2-bis(trimethylsilyl)cytosine (prepared of 0.6 g of cytosine) and II (2.1 g) in dry 1,2-dichloroethane (50 ml) a solution of stannic chloride (1 ml) in 1,2-dichloroethane (8 ml) was added and the mixture was heated at reflux for 1 hr. The mixture was then cooled, diluted with chloroform (50 ml) and neutralized with the saturated sodium hydrogen carbonate solution (75 ml). The organic layer was separated and the aqueous phase was extracted with chloroform (2 x 30 ml). The combined extracts were dried over Na_2SO_4 and evaporated. The saturated at 0° solution of ammonia in methanol (50 ml) was then added to the residue, the mixture was kept at room temperature overnight and evaporated. The residue was

dissolved in the methanol-water (1:4) mixture, and applied to the Dowex-50 (H^+) column (2.5 x 4 cm). The resin was washed with water and the substance was eluted with a 3% solution of ammonium hydroxide. The solution was evaporated to dryness, dissolved in a minimal amount of ethanol and poured into ether (50 ml). The residue was filtered off, dissolved in water and freeze-dried. The yield of VI was 1.0 g (72.5%). Anal. Calcd. for $C_9H_{12}N_6O_4$ (268.23): 40.29%C, 4.47%H, 31.33%N; found: 40.18%C, 4.51%H, 31.20%N.

3'-Azido-3'-deoxyuridine (IX)

To a solution of 2,4-bis(trimethylsilyl)uracil (prepared of 1.35 g of uracil) and II (2.9 g) in dry acetonitrile (75 ml) a solution of stannic chloride (1.3 ml) in dry 1,2-dichloroethane (2 ml) was added and the mixture was heated at reflux for 1.5 h. The solvents were then evaporated, chloroform (200 ml) and saturated solution of sodium hydrogen carbonate (100 ml) were added to the residue and the mixture was filtered through a layer of Super-cel Hyflo. The organic layer was separated, the water layer was extracted with chloroform (50 ml). The extracts were combined, dried over Na_2SO_4 and evaporated to dryness. A saturated at 0° solution of ammonia in methanol (75 ml) was then added to the residue, the mixture was kept at room temperature overnight, and evaporated to dryness. The residue was dissolved in a mixture ethanol-water (1:1) and applied to the Dowex 1 x 4 (OH^-) column (2.5 x 7 cm). The resin was washed with a 50% aqueous ethanol and the substance was eluted with 0.1 M solution of ammonium hydrogen carbonate. The fractions, containing IX, were combined, evaporated and the residue was several times co-evaporated with water, dissolved in water (30 ml) and freeze-dried. The yield of IX was 2.1 g (97%). Anal. Calcd. for $C_9H_{11}N_5O_5$ (269.22): 40.15%C, 4.12%H, 26.02%N; found: 40.13%C, 4.16%H, 29.98%N.

3'-Amino-3'-deoxyadenosine (VII)

Method A. A 2 M solution of sodium methylate in methanol (60 ml) was added to III (3.0 g) and the mixture was heated at

reflux for 24 hrs. The mixture was cooled, neutralized with Dowex-50 (H^+) and was applied to the column, containing additional 10 ml of Dowex-50 (H^+). The resin was washed with 100 ml of water and the product was eluted with 3% NH_4OH in water. The solution was evaporated to dryness and the residue was washed with hot ethanol, then with ether and dried in vacuo. The yield of VII was 1.1 g (43%), m.p. 260° (lit. [16] 260°).

Method B. To a solution of V (2 g) in pyridine (60 ml) triphenylphosphine (10 g) was added and the mixture was kept for 3 hrs at room temperature. A 25% aqueous NH_4OH (40 ml) was then added to the mixture, it was kept at room temperature for 2 hrs and evaporated to dryness. The residue was washed several times with hot ethanol, then with ether and dried in vacuo. The yield of VII was 1.65 g (90.5%), m.p. 260° (lit.[12] 260°). Anal. Calcd. for $C_{10}H_{14}N_6O_3$ (266.26): 45.11%C, 5.30%H, 31.57%N; found: 44.93%C, 5.48%H, 31.31%N.

3'-Amino-3'-deoxycytidine (VIII)

Method A. A 2 M solution of sodium methylate in methanol (30 ml) was added to IV (1.7 g) and the mixture was heated at reflux for 24 hrs. The mixture was cooled, neutralized with Dowex-50 (H^+) and applied to the column, containing additional 5 ml of Dowex-50 (H^+). The resin was washed with 100 ml of water and the products were eluted with a 3% NH_4OH. The solution was evaporated to dryness and the residue dissolved in 2 ml of water and applied to the silica gel column (2.5x20 cm), equilibrated with the system ethanol-n-butanol (3:1). The elution was run at first with 150 ml of the same system, the 3 ml fractions being collected. The coloured fractions were removed, and the colourless, containing IV combined. The further elution (using 150 ml of ethanol) gave the residual IV. All the fractions containing IV were combined and evaporated to dryness. The recovery of IV was 0.31 g. The compound VIII was eluted with 250 ml of a 85% aqueous ethanol. The solution was evaporated to dryness. The residue was dissolved in water (250 ml) and freese-dried. The yield of VIII was 0.56 g (39%).

Method B. To a solution of VI (268 mg) in pyridine (4 ml) triphenylphosphine (1.5 g) was added and the mixture was kept

at room temperature for 3 hrs. A 25% aqueous NH_4OH (2 ml) was then added to the mixture, which was kept at room temperature for additional 2 hrs and finally evaporated to dryness. Ether (50 ml) and water (50 ml) were then added to the residue, the aqueous layer was separated and evaporated to dryness. The residue was dissolved in minimal amount of ethanol and the solution was added dropwise into 80 ml of ether. The residue was filtered off, dissolved in water (25 ml) and freeze-dried. The yield of VIII was 184 mg (76%). Anal Calcd. for $C_9H_{14}N_4O_4$ (242.23): 44.62%C, 5.83%H, 23.13%N; found: 44.59%C, 5.86%H, 23.21%N.

3'-Amino-3'-deoxyuridine (X)

To a solution of IX (54 mg) in a mixture of pyridine - 25% aqueous ammonium hydroxide (1:1,2 ml) triphenylphosphine (156 mg) was added, the mixture was kept for 2 hrs at room temperature, and evaporated to dryness. Water (20 ml) and ether (20 ml) were then added to the residue, the water layer was separated, washed with ether (3 x 15 ml), concentrated to a 10 ml volume and freeze-dried. The yield of X was 46 mg (94%). Anal. Calcd. for $C_9H_{13}N_3O_5$ (243.22): 44.44%C, 5.39%H, 17.28%N; found: 34.40%C, 5.42%H, 17.21N.

3'-Azido-3'-deoxynucleoside 5'-phosphates (XXII-XXIV)

A cooled to 0° solution of $POCl_3$ (0.4 ml) in triethylphosphate (20 ml) was added to a nucleoside (2 mmole). The mixture was kept for 24 hrs at 4° and finally neutralized with 25% aqueous ammonium hydroxide and left for additional 1 h at 4°. Water (50 ml) was then added, and the mixture was extracted with benzene (25 ml) and then with ether (2 x 25 ml). The water layer was separated, evaporated to dryness and dissolved in water (200 ml). The further isolation of nucleotides was run in the following way. The solutions of XXII and XXIII in water were applied to Dowex-50 (H^+) columns (2.5 x 10 cm). The elution was performed using water. The UV-absorbing fractions were collected, combined, evaporated to dryness, co-evaporated with a 10% aqueous ammonium hydroxide (15 ml) dissolv-

ed in water (25 ml) and freeze-dried. The yield of XXII was 73%. The yield of XXIII was 58%.

The solution of XXIV in water was applied to the DE-32, column (5 x 25 cm). The elution was performed with a linear gradient of concentration (0.0 M → 0.2 M, V = 7.5 l) of ammonium hydrogen carbonate buffer. The fractions containing XXIV (the 0.11 M concentration of ammonium hydrogen carbonate) were combined, evaporated to dryness, several times co-evaporated with water and freeze-dried. The yield of XXIV was 39%.

3'-Amino-3'-deoxynucleoside 5'-phosphates (XXV-XXVII)

To a solution of azidonucleotide (XXII-XXIV, 1 mmole) in the mixture of pyridine (15 ml) and a 25% aqueous ammonium hydroxide (10 ml) triphenylphosphine (800 mg) was added and the mixture was kept overnight at room temperature. The solvents were then evaporated to dryness, water (50 ml) and ether (50 ml) were added to the residue. The water layer was separated, washed with ether (2 x 25 ml), concentrated to a 20 ml volume and freeze-dried. Yields: 97% of XXV, 83% of XXVI, 84% of XXVII.

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