A General Synthesis of N-Glycosides. V.[1,2] Synthesis of 5-Azacytidines

U. Niedballa and H. Vorbrüggen*

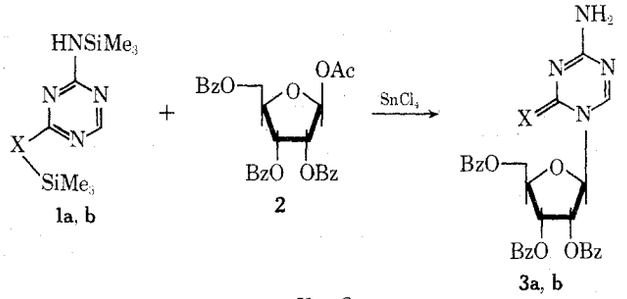
Research Laboratories of Schering A.G., Department Allegemeine Synthetische Chemie, 1 Berlin 65, Muellerstrasse 170–172, Germany

Received March 29, 1974

Reaction of silylated 5-azacytosines as well as their silylated 2-thio analogs with protected 1-*O*-acyl sugars in the presence of $SnCl_4$ gave the corresponding 5-azacytidines in good yields.

The cancerostatic 5-azacytidine, which is highly active against leukemia,[3] and certain analogs were first prepared *via* a multistep synthesis starting from peracetylated 1-glycosyl isocyanates by Píškala and Šorm.[4] Subsequently, 5-azacytidine was isolated as a new antibiotic by Hanka, *et al.*,[5] from *Streptoverticillium ladakanus*. More recently Winkley and Robins[6] treated silylated 5-azacytosines with acylated 1-halo sugars but obtained only fair yields of 5-azacytidine and its 2′-deoxy and other analogs.

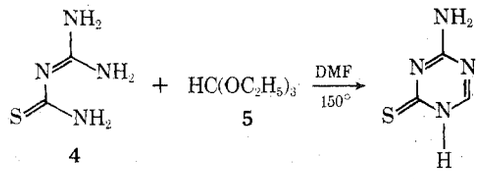
The biological importance of 5-azacytidine[3] induced us to apply our new Friedel–Crafts catalyzed silyl Hilbert–Johnson procedure[7] to the synthesis of 5-azacytidines. Reaction of silylated 5-azacytosine (**1a**) and 2-thio-5-azacytosine (**1b**) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**2**) in 1,2-dichloroethane or acetonitrile in the presence of $SnCl_4$ gave the corresponding O-benzoylated 5-azacytidines **3a** and **3b** in yields of up to 80%, thus making these interesting compounds readily available.[8]



a, X = O
b, X = S

The preparation of the base 5-azacytosine[9] was simplified by direct synthesis from *N*-cyanoguanidine and formic acid–acetic anhydride in 35% yield.

The new 2-thio-5-azacytosine (**6**) was obtained by condensation of thiocarbamoylguanidine (**4**) with ethyl orthoformate (**5**) in dimethylformamide at 150° in analogy to Píškala[9] to give **6** in 72% yield, which could be readily silylated to the crystalline *S,N*-bissilyl compound **1b.**

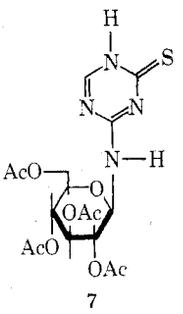


During the reaction of **1b** with pentaacetyl-β-D-glucopyranose (**10**) the N^4-(glucosyl)-2-thio-5-azacytosine (**7**) was isolated as a side product and characterized by uv and nmr spectra. The anomeric H-1′ proton [δ 5.73 (dd, $J = J' \approx 9$ Hz)] is split by the NH group, which disappears on exchange with D_2O to give a doublet [δ 5.72 (d, $J = 9$ Hz)].

Table I
Preparation of Acylated 5-Azacytidine and Analogs

Silylated s-triazine	Acylated sugar	Acylated nucleoside	Yield, %
2,4-*O*,*N*-Bis(trimethylsilyl)-4-amino-1,3,5-triazine (**1a**)	1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**2**)	**3a**	81
	1,2,3,5-Tetra-*O*-acetyl-β-D-ribofuranose (**8**)	**9a**	50
	1,2,3,4,6-Penta-*O*-acetyl-β-D-glucopyranose (**10**)	**11a**	78
	1,2,3,4-Tetra-*O*-acetyl-β-D-ribopyranose (**12**)	**13a**	52
	2-Deoxy-3,5-di-*O*-*p*-toluoyl-α-D-ribofuranosyl chloride, (**14**)	**15a**	42[a]
2,4-*S*,*N*-Bis (trimethylsilyl)-4-amino-2-mercapto-1,3,5-triazine (**1b**)	1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**2**)	**3b**	82
	1,2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranose (**10**)	**11b**	59
	1,2,3,4-Tetra-*O*-acetyl-β-D-ribopyranose (**12**)	**13b**	56

[a] Yield of β anomer, total yield 77%.



The saponification of the O-benzoylated 5-azacytidine (**3a**) to free 5-azacytidine is difficult and best results are obtained following closely the procedure of Píškala and Šorm.[4] However, all attempts to saponify or transesterify the O-benzoylated 2-thio-5-azacytidine (**3b**) and its analogs failed. Apparently the heterocyclic ring in **3b** opens readily under basic conditions. The cleavage is accompanied by a

shift in the uv maxima from 283 to 277 nm and by disappearance of the H-6 proton at δ 8.2 in the nmr spectrum.

In Table I the preparation of acylated analogs of **3** is summarized.

Experimental Section

For instruments and the purification of solvents compare part I[7] of this series.

A. 4-Amino-1,2-dihydro-1,3,5-triazine-2-one. A mixture of 98–100% formic acid (80 ml, 2.12 mol), acetic anhydride (80 ml, 0.816 mol), and *N*-cyanoguanidine (dicyandiamide, Merck, Darmstadt) (84.08 g, 1 mol) was heated to 100°, whereupon the reaction started to boil vigorously. The solid dissolved and after a short time a colorless precipitate separated. The reaction was completed by heating to 140° for 2 hr. After cooling to 22° the solid was filtered and the crude material extracted three times with boiling ethanol to give after drying *in vacuo* a white powder (38.8 g, 34.6%) with mp 350°, which could be readily silylated in high yields according to standard procedures to give **1a**.[9]

Anal. Calcd for $C_3H_4N_4O$ (112.10): C, 32.15; H, 3.60; N, 49.99. Found: C, 31.87; H, 3.69; N, 50.13.

B. 4-Amino-1,2-dihydro-1,3,5-triazine-2-thione (6). A suspension of freshly prepared **4**[10] (118.2 g, 1 mol) and **5** (148.2 g, 1.8 mol) in dry dimethylformamide (500 ml) was refluxed (oil bath, 160°) for 2 hr with exclusion of moisture, whereupon the solid dissolved and after a few minutes a crystalline product separated. After cooling to 22° the crystalline material was filtered, washed with ethanol, and dried *in vacuo* at 50° to give 92.5 g (72%): mp >330°; nmr (NaOD) δ 8.00 (s, 1, H-6); uv (CH_3OH) λ_{max} 210 nm (ϵ 10,900), 270 (16,900).

Anal. Calcd for $C_3H_4N_4S$ (128.16): C, 28.12; H, 3.15; N, 43.72; S, 25.02. Found: C, 27.84; H, 3.31; N, 43.69; S, 24.84.

C. 2,4-*S,N*-Bis(trimethylsilyl)-4-amino-2-mercapto-1,2,5-triazine (1b). **6** (38.45 g, 300 ml) was suspended in a mixture of HMDS (400 ml), pyridine (1.2 l.), and trimethylchlorosilane (1 ml). The mixture was refluxed, whereupon the solid dissolved almost completely. The solution was filtered from the solid under nitrogen and concentrated *in vacuo* to 400 ml. **1b** crystallized from the hot solution and was filtered in an atmosphere of nitrogen and washed with a small amount of absolute benzene. The product was dried *in vacuo* at 50°: yield, 68.2 g (83.7%).

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-4-amino-1,2-dihydro-1,3,5-triazin-2-one (3a). To **2** (5.0 g, 9.91 mmol) and **1a** (12.5 mmol) in 1,2-dichloroethane (150 ml) $SnCl_4$ (1.68 ml, 14.16 mmol) in 1,2-dichloroethane (20 ml) was added at 10°. After stirring at 10° for 2 hr the solution was diluted with CH_2Cl_2 and washed with ice-cold saturated $NaHCO_3$ solution. The organic phase was filtered through a layer of Celite, which was washed with a small amount of CH_2Cl_2. After drying (Na_2SO_4) and evaporation, the residue was dissolved in toluene and filtered through Celite to remove unreacted 5-azacytosine. After evaporation *in vacuo* the residue (5.2 g) was dissolved in ethanol and filtered again through Celite. **3a** crystallized from the filtrate as needles: yield, 4.45 g (80.7%); mp 186–187°; $[\alpha]^{20}D$ −33.1° (*c* 1, $CHCl_3$); nmr ($CDCl_3$) δ 8.21 (s, 1, H-6), 6.1–5.9 (m, 3, H-1′, H-2′, H-3′).

Anal. Calcd for $C_{29}H_{24}N_4O_8$ (556.54): C, 62.59; H, 4.35; N, 10.07. Found: C, 62.43; H, 4.41; N, 10.21.

3a gave on methanolysis[4] 5-azacytidine, mp 232–233° (EtOH) (lit.[4] 230–231° dec).

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-4-amino-1,2-dihydro-1,3,5-triazin-2-one (9a). To **8** (6.36 g, 20 mmol) and **1a** (25 mmol) in CH_3CN (200 ml) $SnCl_4$ (4 ml, 34.2 mmol) in CH_3CN (100 ml) was added at 22°. After 30 min at 22° and work-up[11] crystallization (ethyl acetate) gave 3.68 g (49.7%) of **9a**: mp 160–161°; $[\alpha]^{23}D$ 3.41° (*c* 1.04, $CHCl_3$); nmr ($CDCl_3$) δ 5.82 (d, 1, J = 3.5 Hz, H-1′).

Anal. Calcd for $C_{14}H_{18}N_4O_8$ (270.33): C, 45.41; H, 4.90; N, 15.13. Found: C, 45.55; H, 5.05; N, 15.33.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-amino-1,-2-dihydro-1,3,5-triazin-2-one (11a). To **10** (15.56 g, 40 mmol) and **1a** (50 mmol) in CH_3CN (300 ml) $SnCl_4$ (8.5 ml, 72.65 mmol) in CH_3CN (150 ml) was added. After 5.5 hr at 22° and work-up[11] recrystallization (ethyl acetate–pentane) gave 13.32 g (77.6%) of **11a**: mp 213–214°; $[\alpha]^{20}D$ 10.6° (*c* 1, $CHCl_3$); nmr ($CDCl_3$) δ 8.21 (s, 1, H-6), 5.98 (d, 1, J = 9 Hz, H-1′).

Anal. Calcd for $C_{17}H_{22}N_4O_{10}$ (442.40): C, 46.15; H, 5.01; N, 12.67, Found: C, 46.31; H, 5.19; N, 12.59.

1-(2,3,4-Tri-*O*-acetyl-β-D-ribopyranosyl)-4-amino-1,2-dihydro-1,3,5-triazin-2-one (13a). To **12** (3.18 g, 10 mmol) and **1a** (12.5 mmol) in 1,2-dichloroethane (150 ml) $SnCl_4$ (1.68 ml, 14.36 mmol) in 1,2-dichloroethane (20 ml) was added. After 2 hr at 22° and work-up[11] the residue (3.14 g) was chromatographed in ethyl acetate on silica gel (200 g). **13a** crystallized from ethanol: yield, 1.92 g (51.9%); mp 128–136° (solvated); $[\alpha]^{20}D$ 30.6° (*c* 1, $CHCl_3$); nmr ($CDCl_3$) δ 6.11 (d, 1, J = 10 Hz, H-1′).

Anal. Calcd for $C_{14}H_{18}N_4O_8$ (370.33): C, 45.41; H, 4.90; N, 15.13. Found: C, 45.26; H, 5.03; N, 15.02 [after drying for 2 hr at 50° (10^{-3} mm)].

1-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-ribofuranosyl)-4-amino-1,2-dihydro-1,3,5-triazin-2-one (15a). To **14** (3.89 g, 10 mmol) and **1a** (12.5 mmol) in 1,2-dichloroethane (150 ml) $SnCl_4$ (0.84 ml, 7.18 mmol) in 1,2-dichloroethane (20 ml) was added. After 2 hr at 22° and work-up[11] crystallization (toluene) afforded a mixture of the anomeric nucleosides (3.55 g, 76.6%) from which **15a** was obtained by fractional crystallization (ethyl acetate): yield, 1.93 g (41.6%); mp 196°; $[\alpha]^{20}D$ 23.7° (*c* 1, $CHCl_3$); nmr ($CDCl_3$) δ 8.37 (s, 1, H-6), 6.27 (dd, 1, J = 8 + 6 Hz, H-1′).

Anal. Calcd for $C_{24}H_{24}N_4O_6$ (464.49): C, 62.06; H, 5.21; N, 12.06. Found: C, 62.35; H, 5.38; N, 12.08.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-4-amino-1,2-dihydro-1,3,5-triazine-2-thione (3b). To a suspension of **1b** (1.64 g, 6 mmol) in CH_3CN (50 ml) $SnCl_4$ (1.6 ml, 13.7 mmol) in CH_3CN (50 ml) was added. After addition of **2** (2.522 g, 5 mmol) and 30 min at 22°, work-up[11] gave crude **3b** (2.86 g), which crystallized as needles (ethyl acetate): yield, 2.34 g (81.7%); mp 201–203°; $[\alpha]^{20}D$ −24.2° (*c* 1, $CHCl_3$); nmr ($CDCl_3$) δ 8.52 (s, 1, H-6), 7.15 (d, 1, J = 3 Hz, H-1′).

Anal. Calcd for $C_{29}H_{24}N_4O_7S$ (572.61): C, 60.83; H, 4.23; N, 9.79; S, 5.60. Found: C, 60.75; H, 4.34; N, 9.87; S, 5.49.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-amino-1,-2-dihydro-1,3,5-triazine-2-thione (11b). To a suspension of **1b** (3.26 g, 11.9 mmol) in CH_3CN (150 ml) $SnCl_4$ (3.2 ml, 27.4 mmol) in CH_3CN (70 ml) was added (**1b** dissolved), followed by **10** (3.89 g, 10 mmol). After 30 min at 22° and work-up[11] **11b** crystallized (ethanol) as colorless needles: yield, 2.72 g (59.4%); mp 246–247°; $[\alpha]^{20}D$ 20.8° (*c* 1, $CHCl_3$); nmr ($CDCl_3$) δ 8.37 (s, 1, H-6), 7.10 (d, 1, J = 9 Hz, H-1′).

Anal. Calcd for $C_{17}H_{22}N_4O_9S$ (458.46): C, 44.54; H, 4.84; N, 12.22; S, 6.99. Found: C, 44.37; H, 4.99; N, 12.30; S, 6.88.

Reaction in 1,2-dichloroethane gave 38% **11b.** From the mother liquor the N_4-glucoside **7** was isolated by preparative tlc (silica gel, ethyl acetate): yield, 213 mg (3%); amorphous; $[\alpha]^{23}D$ 7.1° (*c* 0.48, ethyl acetate); nmr ($CDCl_3$) δ 8.45 (s, 1, H-6), 5.73 (dd, 1, $J = J'$ = 9 Hz, H-1′).

Anal. Calcd for $C_{17}H_{22}N_4O_9S$ (458.46): C, 44.54; H, 4.84; N, 12.22; S, 6.99. Found: C, 44.38; H, 4.96; N, 12.16; S, 7.11.

1-(2,3,4-Tri-*O*-acetyl-β-D-ribopyranosyl)-4-amino-1,2-dihydro-1,3,5-triazine-2-thione (13b). To **1b** (3.26 g, 11.9 mmol) in CH_3CN (100 ml) $SnCl_4$ (3.2 ml, 27.4 mmol) in CH_3CN (70 ml) was added (**1b** dissolved), followed by **12** (3.18 g, 10 mmol). After 1 hr at 22° and work-up[11] **13b** crystallized (ethanol) as needles: yield, 2.08 g (56.2%); mp 237–239°; $[\alpha]^{20}D$ 72.0° (*c* 1, $CHCl_3$); nmr ($CDCl_3$) δ 8.23 (s, 1, H-6), 7.23 (d, 1, J = 9 Hz, H-1′).

Anal. Calcd for $C_{14}H_{18}N_4O_7S$ (386.40): C, 43.52; H, 4.70; N, 14.50; S, 8.30. Found: C, 43.41; H, 4.78; N, 14.56; S, 8.26.

Acknowledgment. We are indebted to Dr. G.-A. Hoyer and Dr. D. Rosenberg for the nmr spectra and Diplom-Ing. G. Huber for the analyses.

Registry No.—**1a,** 52523-35-0; **1b,** 35782-62-8; **2,** 6974-32-9; **3a,** 28998-36-9; **3b,** 29845-69-0; **4,** 2114-02-5; **5,** 122-51-0; **6,** 36469-86-0; **7,** 52523-36-1; **8,** 13035-61-5; **9a,** 10302-78-0; **10,** 604-69-3; **11a,** 29845-67-8; **11b,** 30009-99-5; **12,** 4049-34-7; **13a,** 30370-22-0; **13b,** 30370-25-3; **14,** 4330-21-6; **15a,** 10302-79-1; 4-amino-1,2-dihydro-1,3,5-triazin-2-one, 931-86-2.

References and Notes

(1) Synthesis of Nucleosides No. 13. For a preliminary publication, compare H. Vorbrüggen and U. Niedballa, *Tetrahedron Lett.*, 3571 (1970).
(2) Part IV: *J. Org. Chem.*, **39,** 3668 (1974).
(3) (a) J. Veselý and A. Čihák, *Experientia,* **29,** 1132 (1973); (b) H. Karon, L. Sieger, S. Leimbrock, J. Z. Finklestein, M. E. Nesbit, and J. J. Swaney, *Blood,* **42,** 359 (1973).
(4) A. Piskala and F. Šorm, *Collect. Czech. Chem. Commun.,* **29,** 2060 (1964); German Patent 1922702 (1969).

(5) L. J. Hanka, J. S. Evans, D. J. Mason, and A. Dietz, *Antimicrob. Ag. Chemother.*, 619 (1966).
(6) M. W. Winkley and R. K. Robins, *J. Org. Chem.*, **35**, 491 (1970).
(7) Compare part I of this series: U. Niedballa and H. Vorbrüggen, *J. Org. Chem.*, **39**, 3654 (1974).
(8) Kilogram amounts of 5-azacytidine have been prepared using our procedures by Ash-Stevens Inc. (private communication by Professor C. S. Stevens).
(9) A. Piskala, *Collect. Czech. Chem. Commun.*, **32**, 3966 (1967).
(10) "Organic Synthesis," Collect. Vol IV, Wiley, New York, N.Y., 1968, p. 502.
(11) Compare the preparation of **3a**.

Mixed Alkylation (Methylation and Ethylation) of Adenosine by Diazoethane in Aqueous 1,2-Dimethoxyethane[1]

Lee M. Pike, M. Khurshid A. Khan, and Fritz Rottman*

Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824

Received August 13, 1974

Synthesis of 2′-*O*-ethyladenosine by treatment of adenosine with diazoethane in aqueous 1,2-dimethoxyethane produced several unexpected alkylation products. Characterization of the products by several methods, including mass spectrometry of their trimethylsilyl ethers, indicated that methylation was occurring to approximately the same extent as ethylation. Analysis of the reaction conditions employing palmitic acid as an alkyl acceptor implicated the solvent (1,2-dimethoxyethane) as a potential source of the extraneous methyl groups since only ethylation was observed when diethyl ether was employed as an alternate solvent.

The reaction of adenosine and diazomethane in aqueous 1,2-dimethoxyethane has been used extensively to prepare 2′-Am[2] since the 2′-hydroxyl group is preferentially methylated under these conditions.[3-5] When adenosine was treated with diazoethane under similar reaction conditions, several unexpected products were observed. In this paper these products are identified and found to indicate the occurrence of mixed alkylation. Evidence is presented which is consistent with involvement of the solvent (1,2-dimethoxyethane) in the alkylation reaction under these conditions.

Adenosine and diazoethane were combined in a solvent of aqueous 1,2-dimethoxyethane, the reaction was permitted to reach completion, and the products were resolved by ion exchange chromatography.[6] Six prominent uv-absorbing fractions were observed as shown in Table I, whereas only four fractions were observed following alkylation of adenosine with diazomethane.[5]

Some preliminary conclusions concerning the nature of the six fractions may be reached from their relative yields and column retentions. By analogy to the reaction of adenosine and diazomethane, one of the major products would be 2′-Ae while lesser amounts of the 3′-ethyl ether and dialkylated products would be obtained.[3] Furthermore, the degree of retention of nucleosides on the ion-exchange column may be correlated with increasing ionization potential of available ribose hydroxyl groups.[7] Therefore, the expected order of elution is: dialkylation products, 2′-*O*-alkylation products, and finally 3′-*O*-alkylation products. Compounds with both 2′- and 3′-hydroxyl groups available are not eluted under these conditions. On the basis of yield and elution pattern, fractions 3 and 4 may contain 2′-alkyl ethers, one of which should be 2′-Ae, while fractions 5 and 6 may contain 3′-alkyl ethers.

Components of all six fractions were characterized by descending paper chromatography in the four solvent systems described in Table II. Fractions 3, 4, 5, and 6 each gave a single uv-absorbing spot in all four solvent systems and were estimated to be greater than 97% pure. Although the reaction of diazoethane with adenosine was expected to yield only ethylated products, surprisingly, the compound in fraction 4 migrated with 2′-Am in all four systems and the compound in fraction 6 migrated with 3′-Am in all four systems (Table II). The compounds in fractions 3 and 5 migrated faster than 2′-Am or 3′-Am, respectively, and were well resolved from each other by solvent D. These results were consistent with the tentative identification of fraction 3 as 2′-Ae and fraction 4 as 3′-Ae since the 3′-ethyl ether should have been retained longer than the 2′-ethyl ether on the ion exchange column as discussed above.

Both fractions 1 and 2 were resolved into several components by paper chromatography with the four solvents. Analytical studies on the first two column fractions in the comparable methylated adenosine series indicated they

Table I
Fractionation of Alkylated Nucleosides on Bio-Rad AG 1 Column[a]

Fraction no.	Identity	Registry no.	Tubes pooled in each fraction	Recovery, % of adenosine applied
1			12–24	3.8
2			38–44	1.7
3	2′-*O*-Ethyladenosine	52842-98-5	45–58	11.9
4	2′-*O*-Methyladenosine	2140-79-6	68–81	9.8
5	3′-*O*-Ethyladenosine	52928-62-8	105–120	3.8
6	3′-*O*-Methyladenosine	10300-22-8	179–210	2.8

[a] The column consisted of Bio-Rad AG 1-X2 (OH^-), 200–400 mesh, 4 × 40 cm, equilibrated with 40% ethanol prior to use. The crude reaction mixture (95,100 A_{260} units) was applied in 40% ethanol and eluted with 40% ethanol at a flow rate of 2 ml/min; the tube volume was 20 ml.