25. Nucleotides

Part XXVI[1])

A New Synthesis of 9-(β-D-Ribofuranosyl)uric Acid and its 5'-Monophosphate

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Syntheses for 9-(β-D-ribofuranosyl)uric acid (**16**) and its 5'-monophosphate **14** have been achieved starting from guanosine and applying the 2-(*p*-nitrophenyl)ethyl group for protection of the aglycon moiety as well as the phosphate function. A more efficient and direct approach to **14** uses O^6,O^8-dibenzyl protection and phosphorylation by the *Yoshikawa* procedure. The various protected intermediates have been characterized by spectroscopic means and elemental analysis.

1. Introduction. – Naturally occurring uric-acid (= 1*H*-purine-2,6,8(3*H*,7*H*,9*H*)-trione) ribonucleosides and ribonucleotides have so far been isolated from beef blood [2–7] and the bacterium *Lactobacillus plantarum* [8]. The structure assignment of the beef-blood component turned out to be a long-term problem of controversy [4–7]; only recently, it could be proven to be the 3-(β-D-ribofuranosyl)uric acid [9] mixed with a very small amounts of its 5'-monophosphate [10]. The product from the bacterial source, on the other hand, has been recognized as the 9-(β-D-ribofuranosyl)uric acid 5'-monophosphate [8] by means of an enzymatic dephosphorylation and spectroscopic comparisons with synthetic model substances.

Synthetic efforts towards the preparation of these types of uric-acid derivatives have been based on direct glycosylations of uric acid itself which led, on treatment of its tetra(triethylsilyl) derivative with 1-bromo-2,3,5-tri-*O*-benzoyl-D-ribofuranose in presence of $AgClO_4$ to the 3-ribosyluric acid [11], whereas the reaction of the tetra(trimethylsilyl)uric acid with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose under trimethylsilyl-trifluoromethanesulfonate catalysis, interestingly, gave rise to a mixture of the corresponding 7-mono- and 3,7-diribosyl derivatives [12]. An unambiguous route starting from 6-amino-1-(β-D-ribofuranosyl)uracil proved the structure of the resulting 3-(β-D-ribofuranosyl)uric acid [13], and the interconversions derived from guanosine [14] [15] and 8-bromoxanthosine [16], respectively, are a direct proof of the structure of the isomeric 9-(β-D-ribofuranosyl)uric acid. Finally, 8-hydroxyguanosine 5'-monophosphate was transformed analogously into uric-acid 9-ribonucleotide [17] [18], but yields were always

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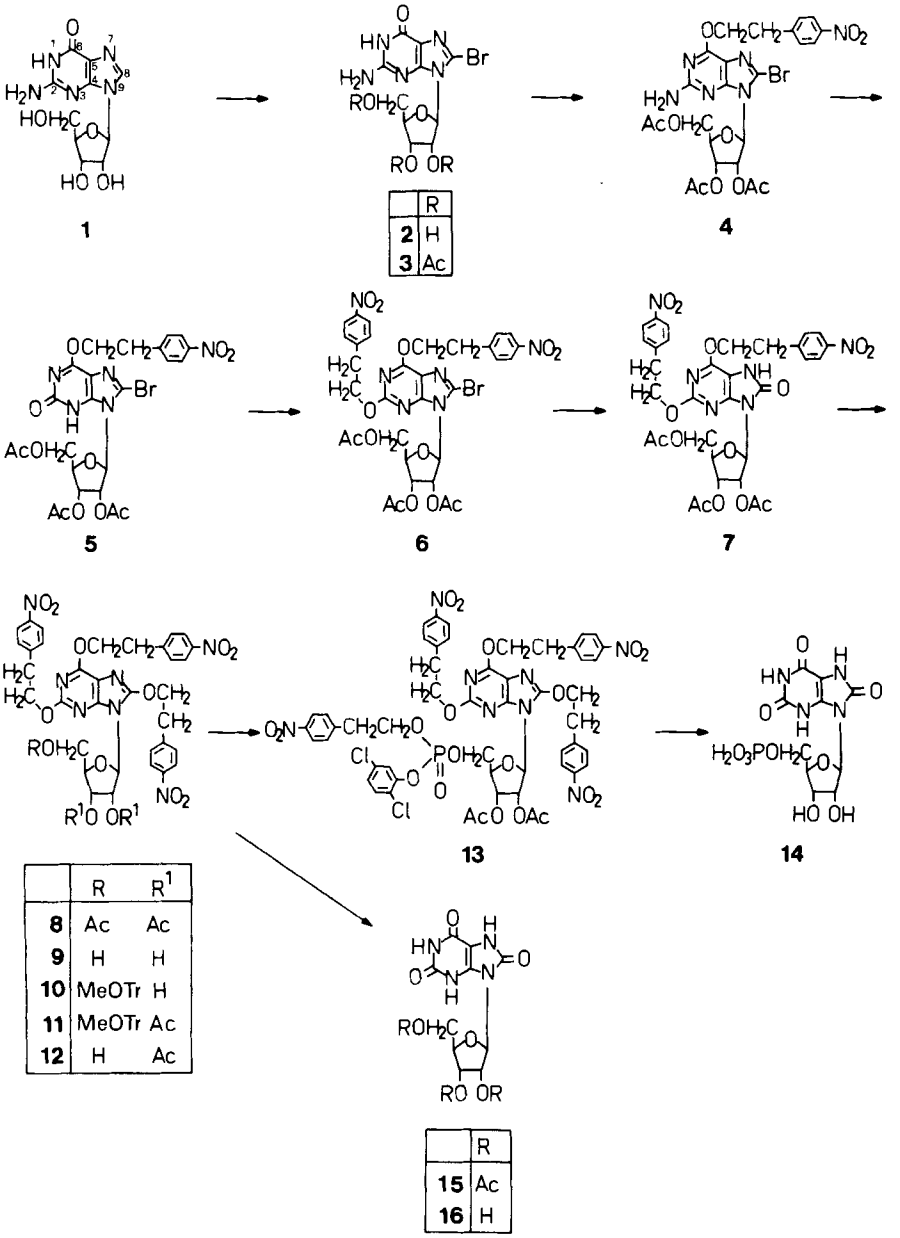
low and all reported processes unsatisfactory from a preparative point of view due to the unusual properties of uric-acid derivatives in general.

2. Syntheses. – Since the encountered synthetic difficulties in this field are primarily due to the three amido functions at the aglycone, we investigated the possibility of

Scheme 1

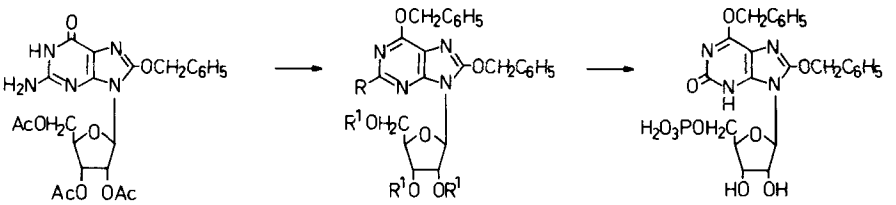


protecting these moieties completely by appropriate blocking groups for better handling of those molecules in general. Guanosine (**1**) was chosen as a cheap starting material and was first converted by known procedures into the 8-bromo derivative **2** followed by acetylation of the ribose moiety to give 2',3',5'-tri-*O*-acetyl-8-bromoguanosine (**3**) in 75% overall yield. The *Mitsunobu* reaction [19] proceeded with **3** and 2-(*p*-nitrophenyl)-ethanol in the usual manner [20] and led in 84% yield to **4**, *i.e.* to O^6-protection [21]. The critical part was then the hydrolytic deamination of the guanosine derivative **4** to the corresponding xanthosine **5**, for which, under optimized conditions with nitrous acid in acetone/H_2O at 10°, a 93% yield could be achieved. O^2-Protection of the amide group in **5** by a silver-ion catalysed alkylation with 2-(*p*-nitrophenyl)ethyl iodide resulted in the formation of the 2,6-bis[2-(*p*-nitrophenyl)-ethoxy]purine **6** in 90% yield. Reflux of this compound in glacial AcOH in presence of NaOAc caused hydrolysis to the 8-keto derivative **7** in 92% yield, and further O^8-alkylation in an analogous silver-ion catalysed process gave the fully protected 9-ribosyluric acid **8** in 88% yield.

Deacetylation at the sugar moiety of **8** to **9** was achieved by short treatment with NaOMe/MeOH at 0° (87% yield). But unexpectedly, cleavage of the 2-(*p*-nitrophenyl)-ethyl groups of **8** (which would give **15**) by β-elimination under various conditions did not work satisfactorily. Thus, 1,5-diazabicyclo[5.4.0]undec-7-en (DBU) in pyridine at room temperature did not lead to the expected product. However, 0.5 N NaOH at 100° removed all blocking groups of the partially deacetylated **12** (see below), to form 9-(β-D-ribofuranosyl)uric acid (**16**) in 72% isolated yield. Unknown side reactions during the β-elimination process are obvious and will be subject of future investigations.

Our first synthesis of 9-(β-D-ribofuranosyl)uric-acid 5'-monophosphate (**14**) resulted from another sequence of reactions including 5'-*O*-monomethoxytritylation of the deacetylation product **9** (see above) to **10** (68%), acetylation to **11** (93%), and detritylation to **12** (98%) by *p*-toluenesulfonic acid in CH_2Cl_2. Phosphorylation of **12** with 2,5-dichlorophenyl phosphorodichloridate and subsequent treatment with 2-(*p*-nitrophenyl)ethanol gave the fully protected uric-acid ribonucleotide **13** in 81% yield. During the final cleavage reactions of all blocking groups by subsequent treatment with oximate [22], ammonia, and 1,5-diazabicyclo[4.3.0]non-5-ene (DBN; better results than with DBU) or with ammonia and DBN, it was again realized that only relatively low yields of the free uric-acid 9-ribonucleotide **14** (41 and 46%, resp.) could be obtained on isolation *via DEAE-Sephadex* ion-exchange chromatography.

Scheme 2



	R	R^1
18	NH_2	Ac
19	OH	Ac
20	OH	H

These experiences prompted us to develop a more satisfying synthesis of **14** starting from 2',3',5'-tri-*O*-acetyl-8-benzyloxyguanosine (**17**) [14] *(Scheme 2)*. The *Mitsunobu* reaction between **17** and benzyl alcohol led again to O^6-alkylation to form 2',3',5'-tri-*O*-acetyl-6,8-dibenzyloxy-9-(β-D-ribofuranosyl)-9*H*-purin-2-amine (**18**), which was stable enough for a hydrolytic deamination with nitrous acid to give the corresponding 2-keto derivative **19** in 77% yield. Deacetylation with NH_3/MeOH gave 6,8-dibenzyloxy-9-(β-D-ribofuranosyl)-3*H*-purin-2(9*H*)-one (**20**), which was then directly phosphorylated in a *Yoshikawa* procedure [23] [24] by $POCl_3$ in trimethyl phosphate at 5° (→**21**). Aqueous workup with Et_3NHHCO_3 buffer and subsequent hydrogenolysis over Pd led to the free uric-acid 9-ribonucleotide **14**, which was isolated as a diammonium salt pentahydrate by ion-exchange chromatography on a *DE-52* cellulose column using a NH_4HCO_3 gradient on elution.

3. Physical Data. – The characterization of the newly synthesized compounds was achieved by the determination of their UV spectra *(Table)*. It is noteworthy that introduction of 2-(*p*-nitrophenyl)ethyl groups sequentially at positions O^6, O^2 and O^8 is associated with a gradual increase of the extinction of the long wavelength absorption band in an additive manner counting the number of the *p*-nitrophenyl chromophors. Compounds possessing acidic H-atoms such as **5**, **7**, **14**, **16**, and **20** have further been subjected to pK_a determinations in order to get an idea about the acidity of these purine derivatives. It is interesting to note in this respect that **5** resembles closely 9-methylxanthine (p*K* 6.12) [25] and **7** and **20** the corresponding 1,3,9- (p*K* 9.39) and 1,7,9-trimethyluric acid (p*K* 5.28) [26], respectively. This agreement indicates that blocking of an amide function in its tautomeric iminol structure does not alter the acidities of such heterocyclic systems very much. Ribosyluric acid **16** and its nucleotide **14** show expectedly closely related properties when compared to 9-methyluric acid [26]. The presence of the 9-(β-D-ribofuranosyl) residue acidifies H–N(3) even stronger by mainly steric reasons. In **14**, the most acidic H-atom is located in the phosphate function but has not been determined exactly since ionization is expectedly not associated with a spectral shift. The monoanion of **14** is, therefore, equivalent to the neutral form of **16**. The deprotonations from the aglycone take place in the sequence N(3), N(7) according to the findings with methylated uric acids [26].

The ^{1}H-NMR spectra are complex due to the various protecting groups, but the sugar protons are located in a distinct region and can, therefore, be used for further characterization. A striking feature of most compounds of this series is seen in the fact that acetylation of the sugar moiety is associated with a low-field shift of H–C(2') even beyond the signal of H–C(1'), whereas usually the reverse order of chemical shifts are observed with acylated ribonucleosides. The presence of bulky substituents at C(8) may be responsible for this spectral change indicating a relatively high population of *syn*-conformers.

Conclusion. – Protection of the amide functions in uric-acid derivatives at the O-atoms causes good solubilities in organic solvents thus facilitating synthetic modifications. By this approach, 9-(β-D-ribofuranosyl)uric-acid 5'-monophosphate (**14**) has been synthesized in preparative scale and has been characterized for the first time analytically in form of its diammonium salt pentahydrate. Fully protected uric-acid ribonucleotides will be valuable components for oligonucleotide syntheses.

Table. *Physical Data of 9-(β-D-Ribofuranosyl)uric-Acid Derivatives*

	pK_4 in H_2O	UV Data[a]) λ_{max} [nm]	lg ε	pH or solvent	Molecular form	^{1}H-NMR chemical shifts [ppm][b]) H–C(1′)	H–C(2′)	H–C(3′)	H–C(4′)	2H–C(5′)	Acetyl	Solvent
3		263 [286]	4.22 [4.15]	MeOH	0	5.87 (*d*)	6.00 (*dd*)	5.64 (*dd*)	4.19–4.44 (*m*)		2.10 (*s*) 2.06 (*s*) 1.98 (*s*)	D_6-DMSO
4		259 285	4.27 4.29	MeOH	0	5.98 (*d*)	6.22 (*t'*)	6.06 (*t'*)	4.30–4.48 (*m*)		2.12 (*s*) 2.09 (*s*) 1.98 (*s*)	$CDCl_3$
5		[250] 270 [277]	[4.15] 4.34 [4.31]	MeOH	0	6.02–6.08 (*m*)		5.78 (*t'*)	4.37–4.48 (*m*)		2.13 (*s*) 2.08 (*s*) 2.04 (*s*)	$CDCl_3$
	6.16	[252] 272 [290]	[4.08] 4.24 [4.07]	4.0	0							
		[260] 286	[4.14] 4.30	9.0	1 –							
6		269	4.48	MeOH	0	6.02 (*d*)	6.24 (*m*)	5.88 (*t'*)	4.11–4.43 (*m*)		2.10 (*s*) 2.08 (*s*) 1.93 (*s*)	$CDCl_3$
7		[218] [244] 276	[4.25] [4.19] 4.49	MeOH	0	6.04 (*d*)	6.10 (*m*)	5.80 (*t'*)	4.14–4.44 (*m*)		2.07 (*s*) 2.04 (*s*) 1.97 (*s*)	$CDCl_3$
	9.88	242 278	4.14 4.49	5.0	0							
		[264] 287	[4.35] 4.45	13.0	1 –							
8		[218] [242] 270	[4.39] [4.31] 4.59	MeOH	0	5.86 (*d*)	5.97 (*m*)	5.73 (*t'*)	4.02–4.35 (*m*)		2.07 (*s*) 2.06 (*s*) 1.95 (*s*)	$CDCl_3$
9		[248] 272	[4.35] 4.60	MeOH	0	5.74 (*d*)	4.73 (*d*)	4.47 (*t'*)	4.24 (*s*)	3.82 (*m*)		$CDCl_3$
10		236 271	4.45 4.59	MeOH	0	5.81 (*d*)	4.95 (*t'*)	4.55 (*t'*)	4.05 (*m*)	3.33 (*m*)		$CDCl_3$
11		235 272	4.48 4.60	MeOH	0	5.84 (*d*)	6.16 (*t*)	5.93 (*t'*)	4.16 (*m*)	3.35 (*m*)	2.06 (*s*) 1.99 (*s*)	$CDCl_3$
12		[220] [244] 272	[4.37] [4.34] 4.61	MeOH	0	5.86 (*d*)	6.00 (*t'*)	5.67 (*dd*)	4.23 (*d*)	3.83 (*m*)	2.15 (*s*) 1.93 (*s*)	$CDCl_3$
13		[218] [230] 271	[4.51] [4.32] 4.61	MeOH	0	5.87–5.95 (*m*)		5.75 (*dd*)	4.15–4.50 (*m*)		2.07 (*s*) 2.06 (*s*)	$CDCl_3$
14		233 286	3.90 4.04	–1.0	0	5.71 (*d*)	4.44 (*m*)	4.19 (*m*)	4.04–4.10 (*m*)			D_2O
	4.56	203 234 286	3.82 3.87 4.04	2.0	1 –							
	11.68	202 238 292	4.33 3.99 4.03	7.0	2 –							
		248 303	4.03 3.96	14.0	4 –							
16	4.55	203 233 287	3.97 3.92 4.05	1.0	0	5.78 (*d*)	4.45 (*m*)	4.31 (*m*)	4.22 (*d*)	3.89 (*s*)		D_2O
	11.01	203 238 293	4.30 4.00 4.04	7.0	1 –							
		249 303	4.04 3.97	14.0	2 –							
18		247 283	4.22 4.01	MeOH	0	5.94 (*d*)	6.10 (*dd*)	5.98 (*t'*)	4.13–4.38 (*m*)		2.07 (*s*) 2.05 (*s*) 1.93 (*s*)	$CDCl_3$
19		237 269 300	4.07 4.04 3.18	MeOH	0	6.04 (*d*)	5.90 (*t'*)	5.45 (*t'*)	4.05–4.29 (*m*)		2.08 (*s*) 2.04 (*s*) 1.95 (*s*)	$CDCl_3$
20		237 272 [300]	4.05 4.01 [3.54]	MeOH	0	5.86 (*d*)	4.95 (*t'*)	4.40 (*t'*)	4.19 (*s*)	3.81 (*dd*)		$CDCl_3$
	6.88	237 [277] 295	4.00 [3.81] 3.92	4.0	0							
		204 242 287	4.61 4.12 4.06	9.0	1 –							

[a]) Values in brackets refer to shoulders.

[b]) *t'* = pseudo-*t*.

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Experimental Part

General. TLC: Precoated silica-gel TLC sheets *F 1500 LS 254* and cellulose TLC sheets *F 1440* from *Schleicher & Schüll.* Prep. TLC: silica gel *60 PF_{254} (Merck)*. Column chromatography: silica gel *Merck 60* (0.063–0.2 mesh). Ion-exchange chromatography: *DE 52* cellulose *(Whatman)*. M.p.: *Büchi* apparatus, model Dr. *Tottoli;* not corrected. UV/VIS: *Cary* recording spectrometer, model *118, Applied Phys. Corp.*, and *Uvikon 820, Kontron;* λ_{max} in nm ($\lg\varepsilon$). ^{1}H-NMR: *Bruker WM 250* in δ (ppm) relative to TMS.

1. *2',3',5'-Tri-*O*-acetyl-2-bromoguanosine* (**3**) [27]. A mixture of abs. pyridine and 20 g (55 mmol) of 8-bromoguanosine [28] is evaporated, then the residue dissolved in 190 ml of abs. pyridine, and Ac_2O (31 ml, 0.33 mol) added. The mixture is stirred for 1 h at r.t., evaporated, the residue dissolved in 300 ml of $CHCl_3$, and then treated with the same volume of an aq. sat. $NaHCO_3$ soln. The $CHCl_3$ layer is dried over Na_2SO_4, evaporated, and coevaporated with toluene. The residue is dissolved in little $CHCl_3$ and purified by silica-gel chromatography (6 × 25 cm; 400 g SiO_2) with a $CHCl_3$/MeOH gradient 19:1 to 9:1. The main fraction gives 25 g (91%) of a colourless amorphous solid. A small amount is crystallized from MeOH to yield **3** as colourless crystals of m.p. 216° ([27]: m.p. 216–218°).

2. *2',3',5'-Tri-*O*-acetyl-8-bromo-*O^6*-[2-(*p*-nitrophenyl)ethyl]guanosine* (**4**). A soln. of 10 g (20.5 mmol) of **3** in abs. dioxane is evaporated. To the residue, 150 ml of abs. dioxane, 6.6 g (25 mmol) of triphenylphosphane, and 3.88 g (23.2 mmol) of 2-(*p*-nitrophenyl)ethanol are added. At r.t. the mixture is treated under stirring for 1 h dropwise with 4.3 g (25.1 mmol) of diethyl azodicarboxylate. After evaporation, the residue is dissolved in 150 ml of Et_2O and chromatographed on silica gel (10 × 10 cm; 300 g SiO_2) with Et_2O. The main fraction is rechromatographed on silica gel (6 × 16 cm; 280 g SiO_2) first with CH_2Cl_2 and then with CH_2Cl_2/EtOAc 4:1. The main fraction gives 11 g (84%) of **4** as a colourless solid. Anal. calc. for $C_{24}H_{25}BrN_6O_{10}$ (637.4): C 45.22, H 3.95, N 13.18; found: C 44.91, H 3.68, N 12.75.

3. *2',3',5'-Tri-*O*-acetyl-8-bromo-*O^6*-[2-(*p*-nitrophenyl)ethyl]xanthosine* (**5**). To a soln. of 2 g (3.1 mmol) of **4** in 50 ml of acetone, 10 ml of H_2O, and 1 ml of 70% $HClO_4$ soln., 0.43 g (6.2 mmol) of $NaNO_2$ in 20 ml of H_2O are added dropwise under stirring at 10°. After 1 h stirring at 10°, the acetone is distilled off and the aq. soln. treated with 100 ml of $CHCl_3$. The $CHCl_3$ layer is dried over Na_2SO_4, evaporated to a small volume, and chromatographed on silica gel (3 × 21 cm; 80 g SiO_2) with $CHCl_3$/MeOH 97:3. The main fraction gives 1.86 g (93%) of **5** as a colourless amorphous solid. Anal. calc. for $C_{24}H_{24}BrN_5O_{11}$ (638.4): C 45.15, H 3.79, N 10.97; found: C 45.13, H 3.81, N 10.51.

4. *2',3',5'-Tri-*O*-acetyl-8-bromo-*O^2*,*O^6*-bis[2-(*p*-nitrophenyl)ethyl]xanthosine* (**6**). A mixture of 9.9 g (15.5 mmol) of **5** and 4.3 g (15.5 mmol) of Ag_2CO_3 is refluxed for 15 min. After addition of 4.45 g (16 mmol) of 2-(*p*-nitrophenyl)ethyl iodide, reflux is continued for 1 h, the insoluble material filtered off, and the filtrate evaporated. The residue is dissolved in little CH_2Cl_2 and chromatographed on silica gel (6 × 30 cm; 500 g SiO_2) with CH_2Cl_2 and then with $CHCl_3$. The main fraction gives, on evaporation, 11 g (90%) of **6** as an amorphous solid. Anal. calc. for $C_{32}H_{31}BrN_6O_{13}$ (787.5): C 48.80, H 3.96, N 10.67; found: C 48.83, H 3.80, N 10.37.

5. *2',3',5'-Tri-*O*-acetyl-2,6-bis[2-(*p*-nitrophenyl)ethoxy]-9-(β-*D*-ribofuranosyl)-7*H*-purin-8(9*H*)-one* (**7**). In 45 ml of AcOH 3.74 g (4.75 mmol) of **6** are dissolved and then 2.4 g (19 mmol) of NaOAc added. The mixture is refluxed for 3 h, the residue obtained on evaporation suspended in $CHCl_3$ and then treated with a sat. $NaHCO_3$ soln. The $CHCl_3$ layer is dried over Na_2SO_4, concentrated to a small volume, and chromatographed on silica gel (3 × 9 cm; 35 g SiO_2) with $CHCl_3$. The main fraction gives 3.16 g (92%) of **7** as an amorphous solid. Anal. calc. for $C_{32}H_{32}N_6O_{11}$ (724.6): C 53.04, H 4.45, N 11.59; found: C 52.94, H 4.44, N 11.19.

6. *2',3',5'-Tri-*O*-acetyl-2,6,8-tris[2-(*p*-nitrophenyl)ethoxy]-9-(β-*D*-ribofuranosyl)-9*H*-purine* (**8**). In 60 ml of $CHCl_3$ and 100 ml of abs. toluene are heated under reflux for 15 min 1.2 g (1.66 mmol) of **7** and 0.46 g (1.66 mmol) of Ag_2CO_3. Then, 0.47 g (1.7 mmol) of 2-(*p*-nitrophenyl)ethyl iodide is added and reflux continued for 6 h. Centrifugation separates the precipitate from the soln. which is decanted and evaporated. The residue is dissolved in little CH_2Cl_2 and chromatographed on silica gel (6 × 36 cm; 600 g SiO_2) first with CH_2Cl_2, then with CH_2Cl_2/$CHCl_3$ 1:1 and finally with $CHCl_3$. The main fraction yields 1.29 g (88%) of **8** as an amorphous solid. Anal. calc. for $C_{40}H_{39}N_7O_{16}$ (873.8): C 54.98, H 4.50, N 11.22; found: C 55.06, H 4.68, N 10.82.

7. *2,6,8-Tris[2-(p-nitrophenyl)ethoxy]-9-(β-D-ribofuranosyl)-9H-purine* (**9**). In a mixture of 7.5 ml of abs. pyridine and 3.6 ml of abs. MeOH, 0.73 g (0.84 mmol) of **8** are dissolved. The soln. is cooled to 0° and 5 ml (2.5 mmol) of 0.5 M NaOMe in MeOH are added and then stirred for exactly 6.5 min. After neutralization with 3 ml of 1M AcOH and evaporation, the residue is dissolved in 50 ml of $CHCl_3$, washed with H_2O, the $CHCl_3$ layer dried over Na_2SO_4, and then again evaporated. Crystallization from 18 ml of EtOH gives 0.543 g (87%) of **9** as colourless crystals of m.p. 156–158°. Anal. calc. for $C_{34}H_{33}N_7O_{13}$ (747.7): C 54.62, H 4.45, N 13.11; found: C 54.38, H 4.39, N 12.92.

8. *5'-O-Monomethoxytrityl-2,6,8-tris[2-(p-nitrophenyl)ethoxy]-9-(β-D-ribofuranosyl)-9H-purine* (**10**). A mixture of abs. pyridine and 1.53 g (2 mmol) of **9** is evaporated and the residue dissolved in 20 ml of abs. pyridine. To this, 0.77 g (2.5 mmol) of 4-methoxytrityl chloride is added and then stirred for 24 h at r.t. The reaction is stopped by addition of 2 ml of MeOH. After evaporation, the residue in 50 ml of $CHCl_3$ is treated with 20 ml of phosphate buffer (pH 7). The org. layer is dried over Na_2SO_4, evaporated and coevaporated with toluene. The residue is dissolved in little CH_2Cl_2 and chromatographed on a short silica-gel column (30 g SiO_2) subsequently with CH_2Cl_2, $CH_2Cl_2/CHCl_3$ 1:1, and $CHCl_3$. The main fraction gives 1.98 g (95%) of **10** as an amorphous solid. Anal. calc. for $C_{54}H_{49}N_7O_{14}$ (1020.0): C 63.58, H 4.84, N 9.61; found: C 63.52, H 5.16, N 9.45.

9. *2',3'-Di-O-acetyl-5'-O-monomethoxytrityl-2,6,8-tris[2-(p-nitrophenyl)ethoxy]-9-(β-D-ribofuranosyl)-9H-purine* (**11**). To a soln. of 1.475 g (1.45 mmol) of **10** in 20 ml of abs. pyridine, 0.55 ml (0.8 mmol) of Ac_2O are added, and the mixture is stirred at r.t. for 3 h. After evaporation, the residue is dissolved in 50 ml of $CHCl_3$, treated with a sat. $NaHCO_3$ soln., the org. layer dried over Na_2SO_4, and then again evaporated. The residue is dissolved in little CH_2Cl_2 and chromatographed on silica gel (20 g SiO_2) with CH_2Cl_2 followed by $CH_2Cl_2/CHCl_3$ 1:1. On evaporation, 1.49 g (93%) of **11** as an amorphous solid is obtained. Anal. calc. for $C_{58}H_{53}N_7O_{16}$ (1104.1): C 63.09, H 4.83, N 8.88; found: C 62.89, H 4.85, N 8.67.

10. *2',3'-Di-O-acetyl-2,6,8-tris[(p-nitrophenyl)ethoxy]-9-(β-D-ribofuranosyl)-9H-purine* (**12**). To a soln. of 1.33 g (1.2 mmol) of **11** in 7 ml of CH_2Cl_2, 20 ml of 2% TsOH in CH_2Cl_2 are added and stirred at r.t. for 1 h. After dilution with 50 ml of $CHCl_3$ and treatment (twice) with phosphate buffer (pH 7), the org. layer is dried over Na_2SO_4. After evaporation, the residue is dissolved in little CH_2Cl_2 and chromatographed on silica gel (20 g SiO_2) with CH_2Cl_2, $CH_2Cl_2/CHCl_3$ 1:1, and $CHCl_3$. The main fraction gives 0.984 g (98%) of **12** as an amorphous foam. Anal. calc. for $C_{38}H_{37}N_7O_{15}$ (831.8): C 54.87, H 4.48, N 11.78; found: C 54.86, H 4.32, N 11.54.

11. *2',3'-Di-O-acetyl-2,6,8-tris[2-(p-nitrophenyl)ethoxy]-9-(β-D-ribofuranosyl)-9H-purine 5'-[2,5-Dichlorophenyl 2-(p-Nitrophenyl)ethyl Phosphate]* (**13**). In 3 ml of abs. pyridine, 0.23 g (3.35 mmol) of 1,2,4-triazole and 0.31 g (1.1 mmol) of 2,5-dichlorophenyl phosphorodichloridate are dissolved. After stirring for 10 min at r.t., the soln. is cooled in ice, and then a soln. of 0.832 g (1 mmol) of **12** in 3 ml of abs. pyridine is added dropwise with stirring. Then, the ice-bath is removed and the mixture stirred for 30 min at r.t. Finally, 0.26 g (1.5 mmol) of 2-(*p*-nitrophenyl)ethanol is added and the mixture stirred for 21 h at r.t. After evaporation, the residue is dissolved in 50 ml of $CHCl_3$ and washed with phosphate buffer (pH 7). The org. layer is dried over Na_2SO_4, evaporated, then coevaporated with toluene, and the residue finally dissolved in little CH_2Cl_2 for chromatography on silica gel (3 × 14 cm; 50 g SiO_2) with CH_2Cl_2 and then with $CHCl_3$. The main fraction leads to 0.98 g (81%) of **13** as an amorphous solid. Anal. calc. for $C_{52}H_{47}Cl_2N_8O_2P$ (1205.9): C 51.79, H 3.93, N 9.29; found: C 51.74, H 3.63, N 8.98.

12. *Diammonium [9-(β-D-Ribofuranosyl)-1H-purine-2,6,8(3H,7H,9H)-trione] 5'-Phosphate* (**14**). 12.1. **14** *from* **13**. To a mixture of 1.4 ml of dioxane, 1.4 ml of Et_3N, and 1.4 ml of H_2O, 80 mg (0.066 mmol) of **13** and 0.11 g (0.66 mmol) of 4-nitrobenzaldehyde oxime are added. After stirring for 1 h at r.t., 4 ml of conc. NH_3 are added, and the mixture is kept over night. After evaporation, the solid residue is dissolved in 4 ml of abs. pyridine containing 0.33 g (2.65 mmol) of DBN. After stirring at r.t. for 3 days, the mixture is neutralized with 3 ml of 1 M AcOH and then evaporated. The residue is distributed between H_2O and $CHCl_3$ and the H_2O layer concentrated to a small volume and then put on a *DEAE*-cellulose *(Whatman De-52)* column (1.5 × 19 cm) for purification. Elution with 0.3 M NH_4HCO_3, evaporation of the main fraction, then coevaporation (several times) with H_2O to remove the NH_4HCO_3, and finally lyophilization of the aq. soln. give 11.3 mg (41%) of **14** as a colourless fluffy powder.

12.2. **14** *from* **13**. To a soln. of 4 ml of dioxane and 2 ml of conc. NH_3 are added 80 mg (0.066 mmol) of **13**. Stirring for 24 h at r.t. is followed by evaporation and coevaporation with abs. pyridine. The residue is treated with 0.33 g of DBN in 5 ml of abs. pyridine with stirring for 8 days. Neutralization by 3 ml of 1M AcOH and workup as in *12.1* gives 12.6 mg (46%) of **14** as a powder.

12.3. **14** *from* **20**. A soln. of 1 g (2.1 mmol) of **20** (see *Exper. 16*) in 15 ml of abs. trimethyl phosphate is cooled to 0°. Then, 0.4 ml (4.4 mmol) of $POCl_3$ are added dropwise with stirring and kept in the ice-box at 5° for 5 days.

After hydrolysis and neutralization by addition of 30 ml of 1M Et_3NHHCO_3 buffer, the soln. is concentrated *in vacuo* to 15 ml (containing **21**) and then added to 240 ml of EtOH/H_2O 1:1 in which 0.1 g of PdO has been reduced by H_2 in a shaking apparatus. Catalytic debenzylation is complete after 2 days. The catalyst is filtered off and the filtrate concentrated to a small volume for purification on a *DEAE*-cellulose column (3 × 86 cm) with a 0–1M NH_4HCO_3 gradient. The monophosphate is eluted with 0.2M NH_4HCO_3. Evaporation of the main fraction and several coevaporations with H_2O followed by lyophilization of an aq. soln. yield 0.584 g (67%) of **14** as a colourless powder.

12.4. **14** *from* **16**. In 15 ml of trimethyl phosphate, 1 g (3.33 mmol) of **16** is dissolved by gentle warming. The soln. is cooled to 0°, and then 0.6 ml (6.66 mmol) of $POCl_3$ are added dropwise with stirring. After 1.5 h, another portion of 0.6 ml of $POCl_3$ is added slowly and stirring continued at 0° for 28 h. The mixture is then treated with 45 ml of 1M Et_4NBH_4 buffer and evaporated. The residue is dissolved in little H_2O, put onto a *DEAE-Sephadex-A-25* column (5 × 50 cm) and then treated first with H_2O followed by a Et_3NH HCO_3-buffer gradient (0–1M). The product is eluted with 0.5M Et_3NH HCO_3. Evaporation and several coevaporations with H_2O give a product which is further purified by paper chromatography on cellulose sheets (58 × 60 cm) with i-PrOH/conc. NH_3/H_2O 55:10:35. The main bands yield, on elution with H_2O and lyophilization, 0.57 g (40%) of **14** as its diammonium salt pentahydrate. Anal. calc. for $C_{10}H_{19}N_6O_{10}P \cdot 5\ H_2O$ (504.3): C 23.81, H 5.79, N 16.66; found: C 23.77, H 4.24, N 16.80.

13. *9-β-(*D-*Ribofuranosyl)-1*H*-purine-2,6,8(3*H*,7*H*,9*H*)-trione* (**16**). In 5 ml of dioxane, 0.166 g (0.2 mmol) of **12** are dissolved, and the mixture is treated with 4 ml of 0.5M NaOH at 100° for 1.5 h. On cooling, the soln. is neutralized by 1M AcOH, evaporated, and then the residue taken up in 30 ml of H_2O and extracted with 30 ml of $CHCl_3$. The aq. layer is concentrated to 3 ml, put onto a *DEAE*-cellulose column (1.5 × 19 cm), and eluted with an NH_4HCO_3 gradient of increasing concentration. The main fraction is evaporated, coevaporated several times with H_2O, dissolved in little H_2O and acidified to pH 2 with 1N HCl. On cooling, 43 mg (72%) of **16** as colourless needles precipitate.

14. *2',3',5'-Tri-*O*-acetyl-*O^6,O^8*-dibenzylguanosine* (**18**). To a soln. of 0.76 g (18.9 mmol) of **17** [27], 6.3 g (24 mmol) of triphenylphosphane, and 3.3 g (30 mmol) of benzyl alcohol in 100 ml of abs. dioxane, 4.1 g (24 mmol) of diethyl azodicarboxylate are added. The soln. is stirred at r.t. for 18 h followed by evaporation to a sirup. The residue is dissolved in 400 ml of CH_2Cl_2, 70 g of silica gel are added, and the suspension is again evaporated. The powder is given on a silica-gel column (4.5 × 50 cm; 260 g SiO_2) prepared in Et_2O. Elution with Et_2O/hexane 3:2 gives, from the main fraction, 5.81 g (51%) of **18** as an amorphous solid. Anal. calc. for $C_{30}H_{31}N_5O_9$ (605.6): C 59.49, H 5.16, N 11.56; found: C 59.19, H 4.93, N 11.25.

15. *2',3',5'-Tri-*O*-acetyl-6,8-dibenzyloxy-9-(β-*D*-ribofuranosyl)-3*H*-purin-2(9*H*)-one* (**19**). To a soln. of 4.27 g (7 mmol) of **18** in 100 ml of acetone and 30 ml of H_2O cooled to 0°, 2.4 ml of 70% $HClO_4$ soln. is added, followed by 0.97 g (14 mmol) of $NaNO_2$ in 40 ml of H_2O (dropwise addition). After 15 min stirring and neutralization with $NaHCO_3$ soln., the acetone is distilled off and the remaining aqueous phase extracted with $CHCl_3$. The $CHCl_3$ extract is dried over Na_2SO_4, evaporated to a small volume, and chromatographed on silica gel (3.4 × 18 cm; 100 g SiO_2) with $CHCl_3/CH_2Cl_2$ 4:1 and $CHCl_3$. On evaporation of the main fraction, 3.29 g (77%) of **19** as a colourless solid is obtained. Further purification with a small amount of material is done by prep. TLC with $CHCl_3$/MeOH 49:1. Anal. calc. for $C_{30}H_{30}N_4O_{11}$ (606.6): C 59.40, H 4.98, N 9.24; found: C 59.22, H 5.08, N 8.97.

16. *6,8-Dibenzyloxy-9-(β-*D*-ribofuranosyl)-3*H*-purin-2(9*H*)-one* (**20**). In 70 ml of NH_3/MeOH at r.t., 3.29 g (5.4 mmol) of **19** is stirred for 24 h. The soln. is evaporated and the residue dissolved in $CHCl_3$ and chromatographed on silica gel (3.6 × 4.5 cm; 30 g SiO_2) with $CHCl_3$/MeOH 19:1. The main fraction yields 2.44 g (94%) of **20** as a solid foam, which can be crystallized from little MeOH or CH_2Cl_2/Et_2O to give crystals of m.p. 124–125°. Anal. calc. for $C_{24}H_{24}N_4O_7$ (480.5): C 59.39, H 5.03, N 11.66; found: C 59.77, H 5.03, N 11.65.

REFERENCES

[1] Part XXV: M. Ichiba, R. Charubala, R. S. Varma, W. Pfleiderer, *Helv. Chim. Acta* **1986,** *69,* 1768.
[2] S. R. Benedict, *J. Biol. Chem.* **1951,** *20,* 638.
[3] A. R. Davis, E. B. Newton, S. R. Benedict, *J. Biol. Chem.* **1922,** *54,* 595.
[4] E. B. Newton, A. R. Davis, *J. Biol. Chem.* **1922,** *54,* 603.
[5] R. Falconer, J. M. Gulland, *J. Chem. Soc.* **1939,** 1369.
[6] C. A. Carter, J. L. Potter, *Fed. Proc.* **1952,** *11,* 195.
[7] E. Leone, *Boll. Soc. Ital. Sper.* **1955,** *31,* 622.
[8] D. Hatfield, R. A. Greenland, H. L. Stewart, J. B. Wyngarden, *Biochim. Biophys. Acta* **1964,** *91,* 163.
[9] H. S. Forrest, D. Hatfield, J. M. Lagowski, *J. Chem. Soc.* **1961,** 963.
[10] D. Hatfield, R. R. Rinehart, H. S. Forrest, *J. Chem. Soc.* **1963,** 899.
[11] L. Birkofer, A. Ritter, H. P. Kühltau, *Chem. Ber.* **1964,** *97,* 934.
[12] J. A. Maurins, R. A. Paegle, M. J. Lidaks, E. I. Kvasyuk, P. V. Kuzmichkin, I. A. Mikhailopulo, *Nucleos. Nucleot.* **1986,** *5,* 79.
[13] R. Lohrmann, J. M. Lagowski, H. S. Forrest, *J. Chem. Soc.* **1964,** 451.
[14] R. E. Holmes, R. K. Robins, *J. Chem. Soc.* **1965,** *87,* 1772.
[15] M. Ikehara, H. Tada, K. Muneyama, *Chem. Pharm. Bull.* **1965,** *13,* 1140.
[16] M. Saneyoshi, *Chem. Pharm. Bull.* **1967,** *16,* 1616.
[17] M. Ikehara, E. Ohtsuka, *Chem. Pharm. Bull.* **1963,** *11,* 961.
[18] M. Ikehara, K. Murao, *Chem. Pharm. Bull.* **1968,** *16,* 1330.
[19] O. Mitsunobu, *Synthesis* **1981,** 1.
[20] T. Trichtinger, R. Charubala, W. Pfleiderer, *Tetrahedron Lett.* **1983,** 711.
[21] F. Himmelsbach, B. S. Schulz, T. Trichtinger, R. Charubala, W. Pfleiderer, *Tetrahedron* **1984,** *40,* 59.
[22] C. B. Reese, L. Zard, *Nucleic Acids Res.* **1981,** *9,* 4611.
[23] M. Yoshikawa, T. Kato, *Bull. Chem. Soc. Jpn.* **1967,** *40,* 2849.
[24] M. Yoshikawa, T. Kato, T. Takenishi, *Bull. Chem. Soc. Jpn.* **1969,** *42,* 3505.
[25] W. Pfleiderer, G. Nübel, *Liebigs Ann. Chem.* **1961,** *647,* 155.
[26] W. Pfleiderer, *Liebigs Ann. Chem.* **1974,** 2030.
[27] R. E. Holmes, R. K. Robins, *J. Am. Chem. Soc.* **1964,** *86,* 1242.
[28] R. Shapiro, S. C. Agarwal, *Biochem. Biophys. Res. Commun.* **1966,** *24,* 401.