

## 176. Nucleosides

Part LXI<sup>1)</sup>

### A Simple Procedure for the Monomethylation of Protected and Unprotected Ribonucleosides in the 2'-*O*- and 3'-*O*-Position Using Diazomethane and the Catalyst Stannous Chloride

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Intensive studies on the diazomethane methylation of the common ribonucleosides uridine, cytidine, adenosine, and guanosine and its derivatives were performed to obtain preferentially the 2'-*O*-methyl isomers. Methylation of 5'-*O*-(monomethoxytrityl)-*N*<sup>2</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]-*O*<sup>6</sup>-[2-(4-nitrophenyl)ethyl]-guanosine (1) with diazomethane resulted in an almost quantitative yield of the 2'- and 3'-*O*-methyl isomers which could be separated by simple silica-gel flash chromatography (*Scheme 1*). Adenosine, cytidine, and uridine were methylated with diazomethane with and without protection of the 5'-*O*-position by a mono- or dimethoxytrityl group and the aglycone moiety of adenosine and cytidine by the 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) group (*Schemes 2-4*). Attempts to increase the formation of the 2'-*O*-methyl isomer as much as possible were based upon various solvents, temperatures, catalysts, and concentration of the catalysts during the methylation reaction.

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**1. Introduction.** – The 2'-*O*-methylribonucleosides are found as minor components in ribonucleic acids of various origins [2–7], for example in tRNAs but also in rRNAs, mRNAs, and snRNAs at selected positions [8–15]. The 2'- and 3'-*O*-methylribonucleosides exhibit resistance against enzymatic and basic hydrolysis, phosphorolysis of the glycosidic bond, as well as hydrolysis by phosphomonoesterases, nucleases (like RNase), and nucleotidases (like snake-venom nucleotidase [16] [17]). These enzymatic specificities make the synthesis of 2'-*O*-methyl derivatives very interesting for systems in which the biological resistance of a nucleoside can be attributed to the breaking of the glycosidic bond [18] [19].

*Smith* and *Dunn* [20] isolated 2'-*O*-methylribonucleosides for the first time in 1959 from the RNA of rat liver and wheat germ. And after scientists began to evaluate their biological importance in the beginning of the sixties [2–5], it did not take very long before their first chemical syntheses were reported. In 1965, *Broom* and *Robins* [21] as well as *Furukawa et al.* [22] published the first syntheses of 2'- and 3'-*O*-methylribonucleosides. Today, numerous means of synthesis of 2'- and 3'-*O*-methylribonucleosides have been reported. These approaches can be separated into three major groups.

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1) Selective synthesis of 2'- and 3'-*O*-methylribonucleosides starting from D-ribose: this preparation entails numerous reaction steps, and only a few methods have been described [23–26].

2) Selective synthesis of 2'-*O*-methylribonucleosides by protection of the 5'- and 3'-OH group with the 1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl (TIPDSi) group introduced by *Markiewicz* in 1979 [27] [28]: to avoid methylation of the aglycone moiety, the pyrimidine nucleosides have to be protected at the *N*<sup>3</sup> [29–32] or *O*<sup>4</sup> [33] [34] (uridine) and *N*<sup>4</sup> position (cytidine) [35], respectively, except when using 1,3-benzodithiolium tetrafluoroborate (BDTF) for introducing the 2'-*O*-methyl group [36]. But even a base protection of the purine nucleosides does not protect them from becoming methylated [35] in the aglycone moiety. For this reason, only purine syntheses beginning from 6-chloropurine (adenosine) [35] [37] and 2,6-dichloropurine ribosides (guanosine) [37] using the TIPDSi group have been described. As a result, this procedure is rather tedious for the synthesis of purine nucleosides, and the overall yields are low because of the many reaction steps involved. The highest overall yields for the synthesis of 2'-*O*-methylnucleosides have been reported in the case of uridine where overall yields of over 60% have been reached by introducing several different protecting groups at the *N*<sup>3</sup> position [31] [32]. The 2'-*O*-methylcytidine may be synthesized starting with uridine when using *O*<sup>4</sup> protecting groups [33] [34].

3) Methylation of unprotected ribonucleosides or partially protected ribonucleosides with the 2'- and 3'-OH group free: most efforts have been made using this route, as it is the most direct procedure to obtain the desired 2'-*O*-methylribonucleosides. The problem in this case is that, when methylating ribonucleosides with the 3'-OH group unprotected, the methylation also gives rise to the undesired 3'-*O*-methyl isomer as well as reaction at the aglycone. The step which is usually the most problematic is the separation of the 2'- and 3'-*O*-methyl isomers and possible base-methylated products. *Table 1* shows an overview of the different methylating agents which have been used so far and the isolated yields of the 2'- and 3'-*O*-methylribonucleosides.

The method described by *Robins* and coworkers in 1971 [58] and 1974 [42] using diazomethane as a methylating agent in the presence of stannous chloride (SnCl<sub>2</sub>) for the methylation of unprotected ribonucleosides in MeOH is a facile procedure with which it is possible to methylate all four common ribonucleosides in high yields. That is why we chose this method as the basis for our methylations.

**2. Syntheses. – 2'- and 3'-*O*-Methylguanosine Derivatives.** Due to its insolubility in most organic solvents, the direct methylation of unprotected guanosine is not convenient. Using diazomethane, SnCl<sub>2</sub>, and the solvent DMF, only a 29% combined yield of 2'- and 3'-*O*-methylguanosine could be obtained by *Robins* and coworkers [42] after a tedious isolation. When 2-aminoadenosine rather than guanosine was employed for the methylation in a subsequent publication [44], 2'- and 3'-*O*-methylguanosine in a combined yield of 80% were isolated. The intermediate 2'- and 3'-*O*-methyl derivatives of 2-aminoadenosine were separated on an anion-exchange column and subsequently converted to the corresponding guanine nucleosides by the enzyme adenosine deaminase. *Ekborg* and *Garegg* [53] as well as *Chattopadhyaya* and coworkers [54] isolated the methylated guanine nucleosides in moderate yields by introducing protecting groups *prior* to methylation.

Table 1. Published Methylations of 2'- and 3'-O-Unprotected Ribonucleosides

Ref. <sup>a)</sup>	Methylating agent/ solvent/catalyst	Overall yield [%]								
		A		C		U		G		
		2'	3'	2'	3'	2'	3'	2'	3'	
R. K. Robins [21] (1965)	CH <sub>2</sub> N <sub>2</sub> /H <sub>2</sub> O	41								
R. K. Robins [38] (1966)	CH <sub>2</sub> N <sub>2</sub> /H <sub>2</sub> O								29 <sup>b)</sup>	
Reese [39] (1968)	CH <sub>2</sub> N <sub>2</sub> /H <sub>2</sub> O	16	5	16	2	11 <sup>c)</sup>				
Gin and Dekker [40] (1968)	CH <sub>2</sub> N <sub>2</sub> /H <sub>2</sub> O	38	11							
M. J. Robins [41] (1971)	CH <sub>2</sub> N <sub>2</sub> /H <sub>2</sub> O					33 <sup>d)</sup>		35 <sup>d)</sup>		
M. J. Robins [42] (1974)	CH <sub>2</sub> N <sub>2</sub> /MeOH/SnCl <sub>2</sub>	38	61	74	15	58	28	15	14	
Markiewicz [43] (1975)	CH <sub>2</sub> N <sub>2</sub> /MeOH/SnCl <sub>2</sub>					67		11		
M. J. Robins [44] (1981)	CH <sub>2</sub> N <sub>2</sub> /DMF/SnCl <sub>2</sub>								40 <sup>e)</sup>	40 <sup>e)</sup>
Shugar [45] (1971)	Me <sub>2</sub> SO <sub>4</sub> /base					12		3		9 <sup>f)</sup>
Shugar [46] (1978)	CH <sub>2</sub> N <sub>2</sub> /MeOH/SnCl <sub>2</sub>								7 <sup>g)</sup>	5 <sup>g)</sup>
Moffatt [47] (1974)	1. Bu <sub>2</sub> SnCl <sub>2</sub> , 2. MeI/DMF					70 <sup>h)</sup>				
Yamauchi [48] (1978)	trimethyl phosphate	22	6	28 <sup>h)</sup>						
Yamauchi [49] (1978)	trimethylloxosulfonium hydroxide					43				
Yamauchi [50] (1980)	trimethylsulfonium hydroxide, [Cu(acac) <sub>2</sub> ]	41	37	60	22					
Yamauchi [51] (1980)	trimethylanilinium hydroxide	6	2	24						
Ts'o [52] (1980)	NaH/MeI in DMF	42								
Garegg [53] (1980)	CH <sub>2</sub> N <sub>2</sub> /MeOH/SnCl <sub>2</sub>								13 <sup>i)</sup>	28 <sup>i)</sup>
Chattopadhyaya [54] (1982)	CH <sub>2</sub> N <sub>2</sub> /DMF/SnCl <sub>2</sub>								43 <sup>j)</sup>	
Chattopadhyaya [55] (1986)	1. Bu <sub>2</sub> SnO, 2. CH <sub>2</sub> N <sub>2</sub> /Et <sub>2</sub> O	55 <sup>k)</sup>	20 <sup>k)</sup>					60 <sup>l)</sup>	10 <sup>l)</sup>	
Inoue [35] (1987)	CH <sub>2</sub> N <sub>2</sub> /DMF/SnCl <sub>2</sub>								30 <sup>m)</sup>	
Noe [56] (1991)	NaH/MeI in DMF	25 <sup>n)</sup>	3 <sup>n)</sup>	25 <sup>n)</sup>	5 <sup>n)</sup>	33 <sup>p)</sup>	8 <sup>p)</sup>	29 <sup>q)</sup>	5 <sup>q)</sup>	
Miller [57] (1992)	Me <sub>3</sub> SiCHN <sub>2</sub> /CH <sub>2</sub> Cl <sub>2</sub> /SnCl <sub>2</sub>								21 <sup>k)</sup>	

<sup>a)</sup> Only the senior author is listed.

<sup>b)</sup> Starting from 2-amino-6-chloro-9-(β-D-ribofuranosyl)purine, followed by conversion of the chloro to the oxo group.

<sup>c)</sup> Deamination of 2'-O-methylcytidine with KNO<sub>2</sub>/AcOH.

<sup>d)</sup> Through preparation of 4-methoxy-1-(β-D-ribofuranosyl)pyrimidin-2(1*H*)-one followed by conversion of the methoxy into an oxo or an amino group.

<sup>e)</sup> Through preparation of 2-aminoadenosine followed by deamination with adenosine deaminase; by altering reaction conditions, the amount of 3'-isomer could be increased.

<sup>f)</sup> Deamination of 2'-O-methylcytidine with sodium hydrogensulfite.

<sup>g)</sup> Through preparation of 6-O-ethylguanosine followed by the removal of the ethyl group by alkaline hydrolysis.

<sup>h)</sup> No separation.

<sup>i)</sup> Through silylation of the 5'-OH group with (*tert*-butyl)diphenylsilyl chloride followed by desilylation with *tert*-butylammonium fluoride.

<sup>j)</sup> Starting from *N*<sup>2</sup>-[(*tert*-butyl)benzoyl]-5'-*O*-tritylguanosine, *N*<sup>2</sup>-[(*tert*-butyl)benzoyl]-2'-*O*-methyl-5'-*O*-tritylguanosine was obtained.

<sup>k)</sup> Starting from *N*<sup>6</sup>,5'-*O*-ditrityl-adenosine followed by detritylation.

<sup>l)</sup> Starting from *N*<sup>3</sup>-benzoyl-5'-*O*-trityluridine followed by the removal of the protecting groups.

<sup>m)</sup> Starting from *N*<sup>2</sup>-isobutyryl-5'-*O*-tritylguanosine, *N*<sup>2</sup>-isobutyryl-2'-*O*-methyl-5'-*O*-tritylguanosine was obtained.

<sup>n)</sup> Separation of the isomers after introducing the phenoxyacetyl group in the *N*<sup>6</sup> and the dimethoxytrityl group in the 5'-OH position.

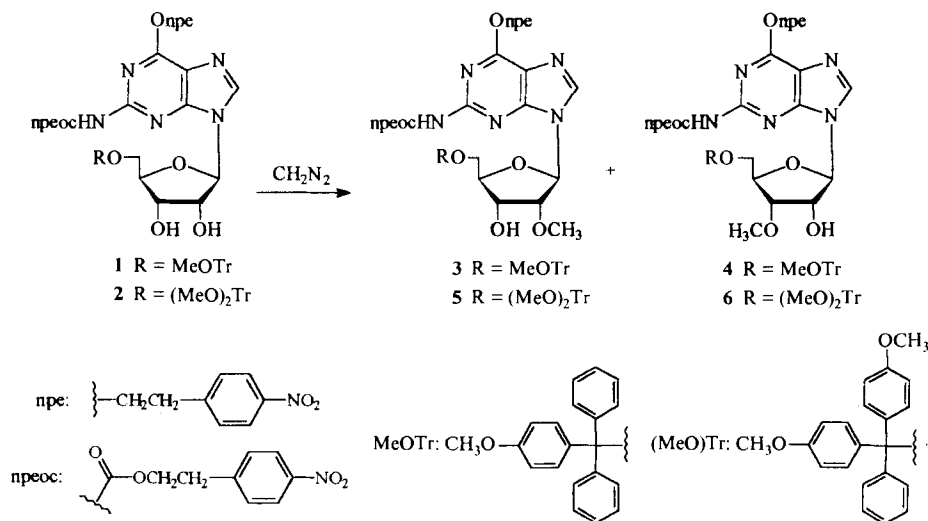
<sup>o)</sup> Separation of the isomers after benzylation and dimethoxytritylation.

<sup>p)</sup> Starting from *N*<sup>3</sup>-(2-cyanoethyl)-5'-*O*-(monomethoxytrityl)uridine, *N*<sup>3</sup>-(2-cyanoethyl)-2'- and 3'-*O*-methyl-5'-*O*-(monomethoxytrityl)uridine were obtained.

<sup>q)</sup> Starting from *O*<sup>6</sup>-[2-(4-nitrophenyl)ethyl]guanosine; separation of the isomers after introducing the phenoxyacetyl group at *N*<sup>2</sup> position and dimethoxytritylation.

In our group, the  $\beta$ -eliminating protecting groups 2-(4-nitrophenyl)ethyl (npe) and 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) are used for the protection of nucleosides with great success. For guanosine, we favor a protection of the amino as well as a protection of the lactam group. The npe group is used for the  $O^6$  and the npeoc group for the  $N^2$  protection. Both groups are stable under mild hydrolytic conditions but can be cleaved quantitatively and simultaneously with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in aprotic solvents during the basic  $\beta$ -eliminating step after oligonucleotide synthesis. Additionally a 5'-OH protecting group normally favors the formation of the 2'-*O*-methyl ether. That is why the methylation was carried out on 5'-*O*-(monomethoxytrityl)- $N^2$ -[2-(4-nitrophenyl)ethoxycarbonyl]- $O^6$ -[2-(4-nitrophenyl)ethyl]guanosine (**1**) or 5'-*O*-(dimethoxytrityl)- $N^2$ -[2-(4-nitrophenyl)ethoxycarbonyl]- $O^6$ -[2-(4-nitrophenyl)ethyl]guanosine (**2**) [59–61] (Scheme 1).

Scheme 1



For the methylation of **1**, a diazomethane solution in 1,2-dimethoxyethane (DME) was used, which was prepared freshly each time from *N*-methyl-*N*-nitrosourea. We investigated the 2':3' ratio as a function of the catalyst and its concentration, the reaction scale, the diazomethane concentration, and the time when it was added, the temperature, and the solvent in which the reaction was carried out. The 2':3' ratio after each methylation was determined by <sup>1</sup>H-NMR. The H-C(1'), H-C(8), and the methyl groups of the 2'-*O*-methyl and 3'-*O*-methyl isomers **3** and **4**, respectively, have different chemical shifts and were used to calculate the 2':3' ratio. The separation of **3/4** was primarily done, after combining several samples, by flash chromatography (silica gel, petroleum ether/CHCl<sub>3</sub>/EtOH). Combined yields were usually *ca.* 95%.

Table 2 shows the dependency of the 2':3' ratio using different metal catalysts. Their choice was suggested by the good results found by *Robins* and coworkers [62] during 2'- and 3'-*O*-monomethylation of adenosine. When using *N,N*-dimethylformamide (DMF)

as solvent,  $\text{SnCl}_2 \cdot 2 \text{H}_2\text{O}$  gave the best results with a 2':3' ratio of 4 to 1. Iron (II or III) chloride gave only a slightly poorer 2':3' ratio, but the formation of additionally methylated products at the base moiety led to difficulties during separation of the isomers on a silica-gel column. The use of  $\text{TiCl}_4$  and  $\text{AlCl}_3$  pushed the 2':3' ratio even further to the 3'-*O*-methyl isomer side. All these metal catalysts showed almost quantitative reaction yields.  $\text{MnCl}_2 \cdot 4 \text{H}_2\text{O}$ ,  $\text{BF}_3 \cdot \text{OEt}_2$ , and  $\text{HBF}_4$  showed only trace amounts of methylated products if any. However, after adding additionally  $\text{SnCl}_2 \cdot 2 \text{H}_2\text{O}$ , the methylation took place in the usual 2':3' ratio, except in the case of the  $\text{HBF}_4$  catalyst, where no methylation occurred after subsequent  $\text{SnCl}_2 \cdot 2 \text{H}_2\text{O}$  addition. Because the metal catalyst  $\text{SnCl}_2 \cdot 2 \text{H}_2\text{O}$  gave the best yield of 2'-*O*-methyl-5'-*O*-(monomethoxytrityl)-*N*<sup>2</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]-*O*<sup>6</sup>-[2-(4-nitrophenyl)ethyl]guanosine (**3**), it was used for all subsequent methylations.

Table 2. 2':3' Ratio for the Methylation of 5'-*O*-(Monomethoxytrityl)-*N*<sup>2</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]-*O*<sup>6</sup>-[2-(4-nitrophenyl)ethyl]guanosine (**1**) Using Different Metal Catalysts at 0°<sup>a)</sup>

Scale [mmol]	Solvent [ml]	CH <sub>2</sub> N <sub>2</sub> Solution			Ratio 2':3' (3/4) <sup>c)</sup>	Catalyst [equiv.]
		Vol [ml]	N <sup>a)</sup>	<i>t</i> [min] <sup>b)</sup>		
1	DMF (50)	2	0.8	5	80:20	SnCl <sub>2</sub> (0.03)
1	DMF (50)	9	0.65	30	76:24 + NMe	FeCl <sub>2</sub> (0.05)
1	DMF (50)	9	0.65	30	78:22 + NMe	FeCl <sub>3</sub> (0.05)
1	DMF (50)	4	0.5	20	67:33	TiCl <sub>4</sub> (0.05)
1	DMF (50)	6	0.5	30	58:42	AlCl <sub>3</sub> (0.05)
1	DMF (50)	6	0.5	30		MnCl <sub>2</sub> (0.05)
					78:22	SnCl <sub>2</sub> (0.02) <sup>d)</sup>
1	DMF (50)	4	0.5	20		BF <sub>3</sub> (0.05)
					80:20	SnCl <sub>2</sub> (0.04) <sup>e)</sup>
1	DMF (50)	8	0.5	30		HBF <sub>4</sub> (1.0)
					no reaction	SnCl <sub>2</sub> (0.04) <sup>f)</sup>

<sup>a)</sup> Normality of the CH<sub>2</sub>Cl<sub>2</sub> solution.

<sup>b)</sup> Time of adding the CH<sub>2</sub>N<sub>2</sub> solution.

<sup>c)</sup> The 2':3' ratio was determined by <sup>1</sup>H-NMR (H-C(1'), H-C(8), and Me groups of the 2'-*O*-methyl and 3'-*O*-methyl isomers have different chemical shifts).

<sup>d)</sup> When there was hardly any reaction perceptible after 5 h of stirring in the presence of MnCl<sub>2</sub>, SnCl<sub>2</sub> was added.

<sup>e)</sup> When there was no reaction perceptible after 2 h of stirring in the presence of BF<sub>3</sub>, SnCl<sub>2</sub> was added.

<sup>f)</sup> Even after adding SnCl<sub>2</sub> and stirring for an additional 5 h, no methylation occurred.

Table 3 shows the dependency of the 2':3' ratio on different reaction conditions applying  $\text{SnCl}_2 \cdot 2 \text{H}_2\text{O}$  as catalyst. There was no dependency of the 2':3' ratio on the concentration of the catalyst, between 6 and 42 mg of  $\text{SnCl}_2 \cdot 2 \text{H}_2\text{O}$ . Neither changing the concentration of the diazomethane solution from 0.05 to 0.8N nor altering the time of addition showed any influence on the 2':3' ratio. When using  $\text{SnCl}_2 \cdot 2 \text{H}_2\text{O}$  as catalyst, there were no *N*-methylated products found, even when the diazomethane solution was added quickly. An excess of 1.5 equiv. of diazomethane was sufficient for complete methylation, and an increase of more than 4 equiv. of diazomethane led to the formation of additional base-methylated products. The scales we used were between 0.25 and 10 mmol of starting material **1**. This had no effect on the 2':3' ratio. Decreasing the

temperature led to a slight increase in the formation of the 2'-*O*-methyl isomer and also decreased the speed of the reaction (below  $-30^{\circ}$ , the reaction was too slow to take place within 24 h). Besides the catalyst, the solvent also had a large impact on the formation of the 2'-*O*-methyl isomer. To accomplish optimum solubility of both, guanine nucleoside and catalyst, mostly polar and aprotic solvents were used. For the methylations in AcOEt, acetone, or MeCN, 15% of *N,N*-dimethylacetamide (DMA) was added for better solubility of the guanine nucleoside and catalyst. When using  $\text{CH}_2\text{Cl}_2$  as solvent, the larger part of the  $\text{SnCl}_2 \cdot 2 \text{H}_2\text{O}$  remained undissolved.

Table 3. 2':3' Ratio for the Methylation of 5'-*O*-(Monomethoxytrityl)- $\text{N}^2$ -[2-(4-nitrophenyl)ethoxycarbonyl]- $\text{O}^6$ -[2-(4-nitrophenyl)ethyl]guanosine (**1**) under Different Reaction Conditions Using  $\text{SnCl}_2 \cdot 2 \text{H}_2\text{O}$  as Catalysts

Scale [mmol]	Solvent [ml]	$\text{CH}_2\text{N}_2$ Solution			Ratio 2':3' (3/4) <sup>e</sup>	$\text{SnCl}_2$ [equiv.]	<i>T</i> [°C]
		Vol [ml]	<i>N</i> <sup>a</sup>	<i>t</i> [min] <sup>b</sup>			
1.0	DMF (50)	2	0.8	5	75:25	0.18	0
1.0	DMF (50)	7	0.5	10	79:21	0.18	0
1.0	DMF (50)	2	0.8	5	79:21	0.075	0
1.0	DMF (50)	2	0.8	50	78:22	0.075	0
1.0	DMF (50)	2	0.8	5	80:20	0.03	0
1.0	DMF (50)	7	0.5	10	75:25	0.04	0
1.0	DMF (50)	25	0.1	8	78:22	0.04	0
1.0	DMF (50)	34	0.05	15	80:20	0.04	0
1.0	DMF (50)	15	0.1	9	80:20	0.04	-10
1.0	DMF (50)	4.5	0.5	2	80:20	0.04	-20
1.0	DMF (50)	2	0.7	1	80:20	0.04	-53 <sup>d</sup> )
1.0	DMF (50)	5.5	0.65	240	83:17	0.04	-30
0.1	DMF (5)	2	0.14	1	75:25	0.04	20
0.25	DMA (10)	0.5	0.7	0.5	82:18	0.04	0
1.4 <sup>e</sup> )	DMA (35)	6	0.4	10	81:19	0.04	-10
10.0 <sup>f</sup> )	DMA (250)	21	0.8	45	83:17	0.04	-10
1.0	DMA (50)	4.5	0.5	2	84:16	0.04	-20
1.0	DMSO (50)	2.5	0.6	5	74:26	0.04	20
0.5	pyridine (25)	2.5	0.6	5	78:22	0.04	0
0.5	$\text{CH}_2\text{Cl}_2$ (100)	4	0.6	5	69:31	0.04	0
0.5	AcOEt (100) <sup>g</sup> )	4	0.6	10	57:34	0.04	0
0.5	acetone (100) <sup>g</sup> )	4	0.6	10	73:27	0.04	0
0.5	MeCN (100) <sup>g</sup> )	4	0.6	10	80:20	0.04	0
0.25	TMU (10)	1	0.8	0.5	83:17	0.04	0
0.25	<i>N</i> -formylpyrrolidine	1.25	0.8	1	78:22	0.04	0
0.25	<i>N</i> -methylpyrrolidin-2-one	0.5	0.8	0.5	77:23	0.04	0
0.25	<i>N</i> -formylmorpholine	0.5	0.8	0.5	76:24	0.04	0
0.25	diethylformamide	2.5	0.8	1	76:24	0.04	0
0.25	<i>N</i> -formylpiperidine	1.5	0.8	1	74:26	0.04	0
0.25	<i>N</i> -methylpiperidin-2-one	0.5	0.8	0.5	75:25	0.04	0
0.25	<i>N</i> -acetylpiperidine	1.5	0.8	1	71:29	0.04	0
0.25	<i>N</i> -acetylmorpholine	0.5	0.8	0.5	67:33	0.04	0

<sup>a</sup>)<sup>b</sup>)<sup>c</sup>) See Table 2. <sup>d</sup>) Because no reaction took place at  $-53^{\circ}$ , the mixture was slowly warmed to room temperature.

<sup>e</sup>) 5'-*O*-(Dimethoxytrityl)- $\text{N}^2$ -[2-(4-nitrophenyl)ethoxycarbonyl]- $\text{O}^6$ -[2-(4-nitrophenyl)ethyl]guanosine (**2** was used instead of **1**); yield: 76% of 2'- and 16% of 3'-*O*-methyl isomer. <sup>f</sup>) Yield: 79% of 2'- and 15% of 3'-*O*-methyl isomer. <sup>g</sup>) For better solubility of starting material and  $\text{SnCl}_2$  DMA (15 ml) was added.

The best 2':3' ratios (83:17) were obtained when the reaction was carried out in *N,N*-dimethylformamide (DMF) at  $-30^{\circ}$ , in DMA at  $-20^{\circ}$ , or in *N,N,N',N'*-tetramethylurea (TMU) at  $0^{\circ}$ . However, the low temperature required for a good 2':3' ratio when using DMF slowed down the normally very fast reaction significantly, and the high-boiling TMU was less convenient on workup. Indeed, after completion of the reaction (TLC control, persistence of the yellow color of diazomethane for a while) the mixture was evaporated before chromatography. When 5'-*O*-(monomethoxytrityl)guanosine **1** (10 mmol) was treated in the described manner with diazomethane at  $-10^{\circ}$  using DMA as solvent, 79% of the 2'-*O*-methyl isomer **3** and 15% of the 3'-*O*-methyl isomer **4** were obtained. Similarly, the 5'-*O*-(dimethoxytrityl)guanosine **2** led to the 2'-*O*-methyl isomer **5** and the 3'-*O*-methyl isomer **6** in 76 and 16% yield, respectively.

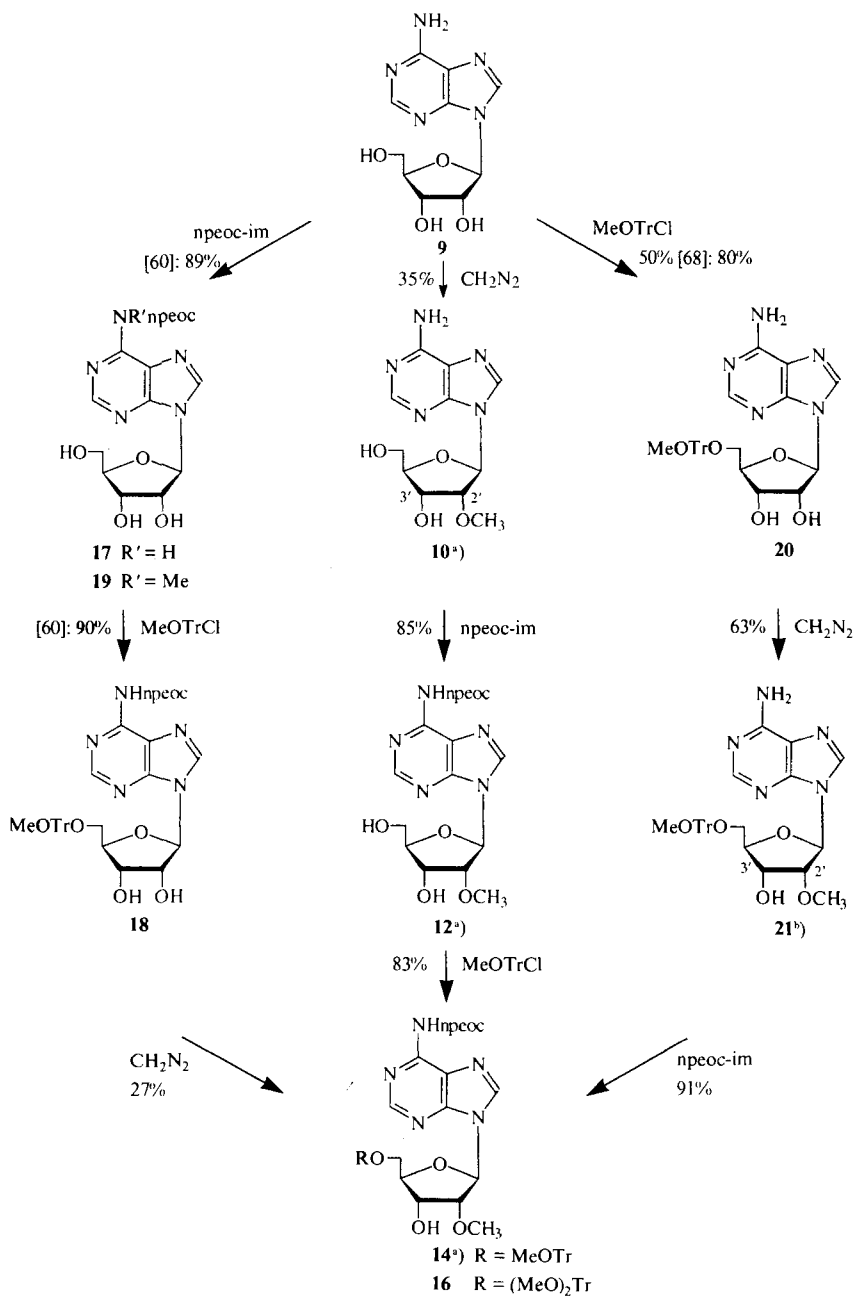
If the 3'-*O*-methyl isomer is the isomer of interest,  $\text{AlCl}_3$  would be the catalyst of choice because here the 2':3' ratio was found to be 58:42, the lowest of all the different conditions applied. The nucleosides **3–6** are properly protected for introducing a phosphoramidite function at the 3'- or 2'-position. With these phosphoramidites, the synthesis of 2'-*O*-methyl-(3'-5')- or 3'-*O*-methyl-(2'-5')-oligoribonucleotides may then be achieved using the npe/npeoc approach.

The unprotected 2'-*O*-methylguanosine (**8**; see *Formula 3*, with R, npe, and npeoc replaced by H) can be obtained from **3** or **5** in two steps by cleavage of the npe and npeoc groups with 1M DBU solution and detritylation. Thus, the intermediate 2'-*O*-methyl-5'-*O*-(monomethoxytrityl)guanosine (**7**; see **3**, with npe and npeoc replaced by H) was formed from **3** in 78% yield and detritylated with 2% TsOH. Despite complete conversion of **7** (TLC), only 60% of **8** was isolated after silica-gel flash chromatography (FC) and subsequent crystallization from  $\text{H}_2\text{O}$ , due to problems of separating TsOH or their salts from the product. Thus, we recommend the use of 80% AcOH for detritylation.

*2'- and 3'-O-Methyladenosine Derivatives.* On methylation of adenosine (**9**) with diazomethane in MeOH, *Robins* and coworkers [42] obtained 2'- and 3'-*O*-methyladenosine (**10** and **11**, resp.) in an almost quantitative combined yield after separation on a *Dowex 1*  $\times$  2 ( $\text{OH}^-$ ) column (*Scheme 2*). The major disadvantage of this approach is that the 2':3' ratio is on the 3' side.

When we performed the methylation of **9** as described, 35% of **10** and 59% of **11** were formed. The target compound for the npe/npeoc strategy being 2'- and 3'-*O*-methyl-5'-*O*-(monomethoxytrityl)-*N*<sup>6</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**14** and **15**, resp.), the already published procedures for adenosine protection were applied [61] [63]. The reaction of **10** and **11** with 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1*H*-imidazol-3-ium chloride (npeoc-im) *via* the transient protection method gave 2'- and 3'-*O*-methyl-*N*<sup>6</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**12** and **13**, resp.) in 85 and 95% yield, respectively. The yield for 2'-isomer **12** was somewhat lower as it did not precipitate out of the reaction mixture and had to be purified from contaminants like triethylammonium salts by FC (silica gel). Attempts to purify **12** by crystallization failed. Treating **12** and **13** with  $\text{MeOTrCl}$  for 72 h in pyridine gave rise to the fully protected 2'- and 3'-*O*-methyl isomers **14** and **15** in 83 and 85% yield, respectively. In an analogous manner, **12** was converted into 2'-*O*-methyl-5'-*O*-(dimethoxytrityl)-*N*<sup>6</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**16**) with dimethoxytrityl chloride ( $(\text{MeO})_2\text{TrCl}$ ). Thus, this is a good method if the 3'-*O*-methyl isomer **15** is the isomer of interest.

Scheme 2



a) The transformation analogous to  $10 \rightarrow 12 \rightarrow 14$  was conducted with the corresponding 3'-O-methyl isomers, *i.e.*  $11 \rightarrow 13 \rightarrow 15$ .

b) The corresponding 3'-O-methyl isomer **22** was also obtained in 31% yield.



However, as we were mainly interested in the 2'-*O*-methyl isomer, we looked for ways to shift the 2':3' ratio to the 2' side. *Robins* and coworkers were unsuccessful in finding an appropriate catalyst for the methylation of adenosine (**9**) which significantly raised the yield of 2'-*O*-methyladenosine (**10**) [62]. Since good solubility of **9** is essential, the solvent cannot be changed much, but introduction of protecting groups *prior* to methylation will help in this case. Therefore, we first methylated 5'-*O*-(monomethoxytrityl)-*N*<sup>6</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**18**; obtained from **17** according to [60] [61]) in DMF with diazomethane (*Scheme 2*). However, this led to *N*<sup>6</sup>,*O*-dimethylated by-products which could not easily be separated from the 2'- and 3'-*O*-methyl isomers **14** and **15**. As *N*<sup>6</sup>-methylation may be of interest in an other context, treatment of **17** in DMF with diazomethane without SnCl<sub>2</sub> catalysis was considered as a direct approach to *N*<sup>6</sup>-methyl-*N*<sup>6</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**19**). Indeed, the latter was obtained in 72% yield from **17**. In contrast, *Sigmund* [64] needed a 5-step synthesis for the preparation of **19** when introducing the *N*<sup>6</sup>-methyl group *prior* to the *N*<sup>6</sup>-npeoc group.

The methylation of 5'-*O*-(monomethoxytrityl)adenosine (**20**) proved to be more promising (*Scheme 2*). The reaction conditions were again altered to increase the formation of the 2'-*O*-methyl isomer **21** over the 3'-*O*-methyl isomer **22** (*Table 4*). Neither changing the size of the scale nor the speed with which the diazomethane solution was added altered the 2':3' ratio. However, adding the diazomethane solution too fast led to additional *N*-methylation at the base, especially with solvents like CH<sub>2</sub>Cl<sub>2</sub> or DMF. The amount of SnCl<sub>2</sub> did not have an impact on the 2':3' ratio. But lowering the amount of catalyst below 0.04 equiv. of the starting material led to a significant reduction of the reaction rate, although without leading to detectable *N*-methylation. To keep **20** in solution, we had to apply polar aprotic solvents. No significant dependence of the 2':3' ratio on the solvent was found, as with the methylation of **1** (see above). Using DMF, DMA, and dimethylsulfoxide (DMSO) led to the same 2':3' ratio (**21/22**) of 66:34. In DMSO, the reaction took place within ½ h, whereas other solvents prolonged the methylation for hours to completion. Therefore, diazomethane in DME was added dropwise to a solution of **20** (15 mmol) and SnCl<sub>2</sub>·2 H<sub>2</sub>O (1 mmol) in DMSO to give 2'-*O*-methyl-5'-*O*-(monomethoxytrityl)adenosine (**21**) and its 3'-*O*-methyl isomer **22** after FC (silica gel) in 63 and 31%, respectively. Thus by performing the methylation on 5'-*O*-(monomethoxytrityl)adenosine (**20**) rather than on adenosine (**9**), the yield of the 2'-*O*-methylated product could be increased from 35 to 63%. Introduction of the npeoc group into **21** and **22** was done in the usual manner leading to 2'- and 3'-*O*-methyl isomers **14** and **15** in 91 and 83%, respectively.

*2'- and 3'-O-Methylcytidine Derivatives.* Diazomethane methylation of cytidine (**23**) is a relatively straightforward method to 2'-*O*-methylcytidine (**24**), but its isolation in 74% yield according to *Robins* and coworkers [42] afforded a time-consuming ion-exchange chromatography for the separation from its 3'-*O*-methyl isomer **25** (*Scheme 3*). On the other hand *Markiewicz* and *Wiewiorowski* [43] reported the isolation of 47% of the 2'-isomer **24** by crystallization. Analogously, from the product obtained under the conditions of *Robins* and coworkers, we were able to isolate 40% of **24** after two crystallizations from EtOH. Additional 29% of **24** and 14% of **25** were obtained from the remaining isomer mixture by ion-exchange on a *Dowex 1 × 2* (OH<sup>-</sup>) column, requiring for a 20-mmol scale almost 2 weeks.

Table 4. 2':3' Ratio for the Methylation of 5'-O-(Monomethoxytrityl)adenosine (**20**) under Different Reaction Conditions (T 20°, if not noted otherwise)

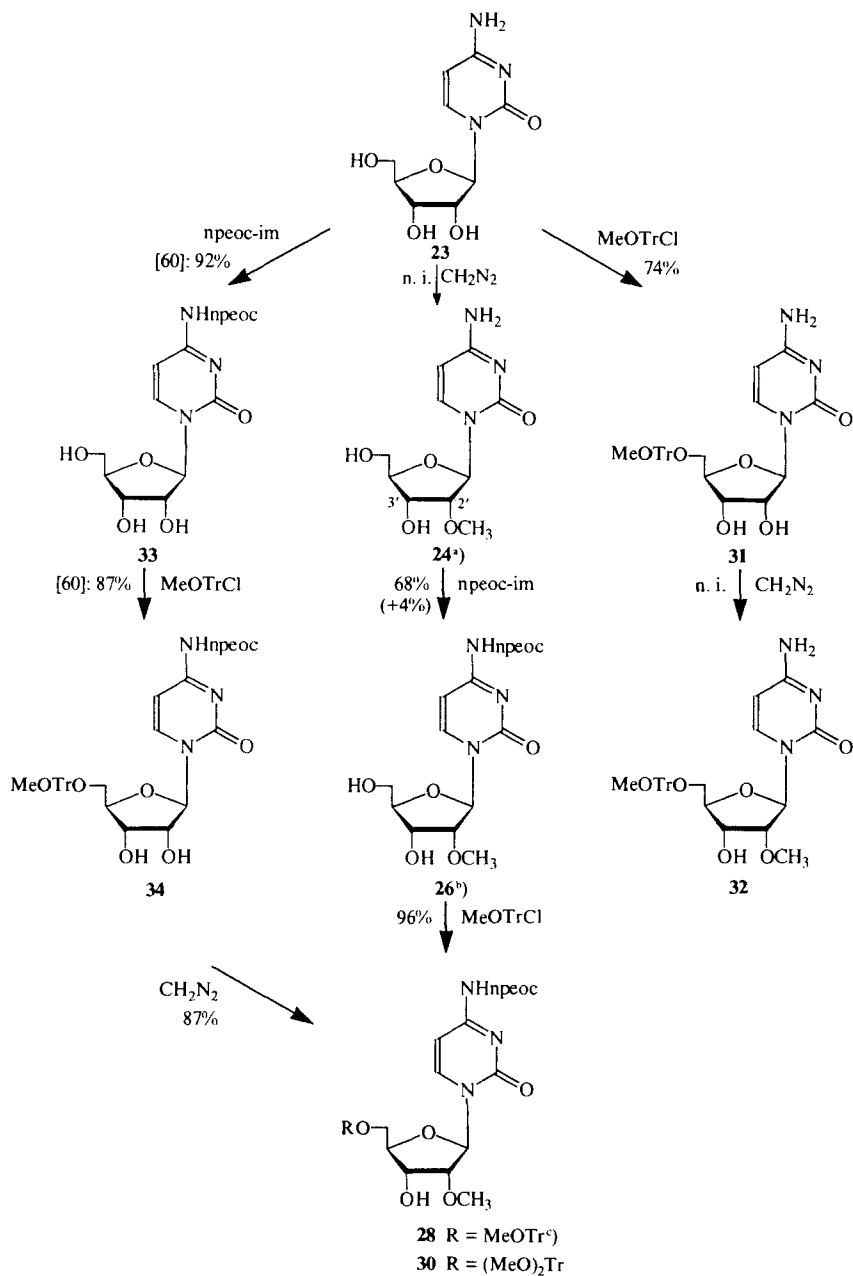
Scale [mmol]	Solvent [ml]	CH <sub>2</sub> N <sub>2</sub> Solution			Ratio 2':3' ( <b>21/22</b> ) <sup>e</sup>	% N- methylation	SnCl <sub>2</sub> [equiv.]
		Vol [ml]	N <sup>a)</sup>	t [min] <sup>b)</sup>			
1.0	DMSO (25)	5	0.45	5	65:35	–	0.1
15.0	DMSO (250)	25	1.0	20	67:33	–	0.066
15.0	DMSO (250)	32	0.8	35	66:34	–	0.066
15.0	DMSO (125)	27	1.0	40	66:34	–	0.033
1.0	DMF (50)/0°	6	0.65	2	66:34	–	0.044
3.7	DMF (75)/0°	7	0.9	5			0.025
		3	0.9	2	68:32	–	0.02 <sup>d)</sup>
15.0	DMF (250)/0°	25	0.75	30	65:35	–	0.066
9.0	DMF (150)	15	0.75	7	58:42	23	0.08
1.0	DMA (30)/0°	3	0.8	2	66:34	–	0.044
5.0	DMA (100)/0°	20	0.9	20			0.022
		5	0.9	10	63:37	1	0.022 <sup>e)</sup>
1.0	TMU (25)	5	0.45	5	59:41	–	0.1
1.0	pyridine (50) <sup>f)</sup>	5	0.45	5	61:39	–	0.1
1.0	CH <sub>2</sub> Cl <sub>2</sub> (5% MeOH)	50	0.1	5	44:56	22	0.072

<sup>a)</sup><sup>b)</sup><sup>c)</sup> See Table 2. <sup>d)</sup> A second amount of SnCl<sub>2</sub>·2 H<sub>2</sub>O and CH<sub>2</sub>N<sub>2</sub> was added after several hours. <sup>e)</sup> A second amount of SnCl<sub>2</sub>·2 H<sub>2</sub>O and CH<sub>2</sub>N<sub>2</sub> was added on the next day. <sup>f)</sup> The solution turned deep red when adding diazomethane solution.

Another approach, applied to the mixture **24/25**, made use of the transient protection method yielding the corresponding *N*<sup>4</sup>-npeoc derivatives according to a procedure published for cytidine [61] [63]. During cleavage of the trimethylsilyl groups, 68% of pure 2'-*O*-methyl-*N*<sup>4</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine (**26**) precipitated out of the reaction mixture (Scheme 3). The remaining 2':3' isomer mixture **26/27** in the mother liquor was best separated after introducing the MeOTr group into the 5'-OH position. Thereby, another 4% of 2'-*O*-methyl-5'-*O*-(monomethoxytrityl)-*N*<sup>4</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine (**28**) and 15% its 3'-*O*-methyl isomer **29** were isolated. By introducing the mono- or dimethoxytrityl group into **26**, the tritylated derivatives **28** and **30** were prepared in 96 and 85%, respectively.

The introduction of the protecting groups *prior* to methylation was also successful in the case of cytidine. Methylation of 5'-*O*-(monomethoxytrityl)cytidine (**31**) in DMF, DMA, or CH<sub>2</sub>Cl<sub>2</sub> (10% of MeOH) at room temperature gave the same 2':3' ratio (*ca.* 4:1) as with cytidine in MeOH (**32** was not isolated), but considerably more base methylation occurred (Scheme 3). However, methylation of 5'-*O*-(monomethoxytrityl)-*N*<sup>4</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine (**34**; obtained from **33** according to [60]) turned out to be the method of choice (Scheme 3). To shift the 2':3' ratio as far as possible to the 2'-side, we changed the scale, the temperature, and the solvent in which the reaction was carried out (see Table 5). The scale and the temperature did not have an impact on the amount of 2'-isomer **28** formed, but different solvents led to different 2':3' ratios. Adding the diazomethane solution quickly led to additional methylation at the base. This depended much on the solvent used as well. The highest yield of 2'-isomer **28** was formed using CH<sub>2</sub>Cl<sub>2</sub> and a 5-mmol-scale reaction: 87% of 2'-*O*-methyl isomer **28** and only 5% of 3'-*O*-methyl isomer **29** could be isolated after FC (silica gel).

Scheme 3



<sup>a)</sup> Obtained as a 82:18 mixture with the minor 3'-O-methyl isomer 25.

<sup>b)</sup> The corresponding 3'-O-methyl isomer 27 was also obtained in 16% yield from 24/25 82:18.

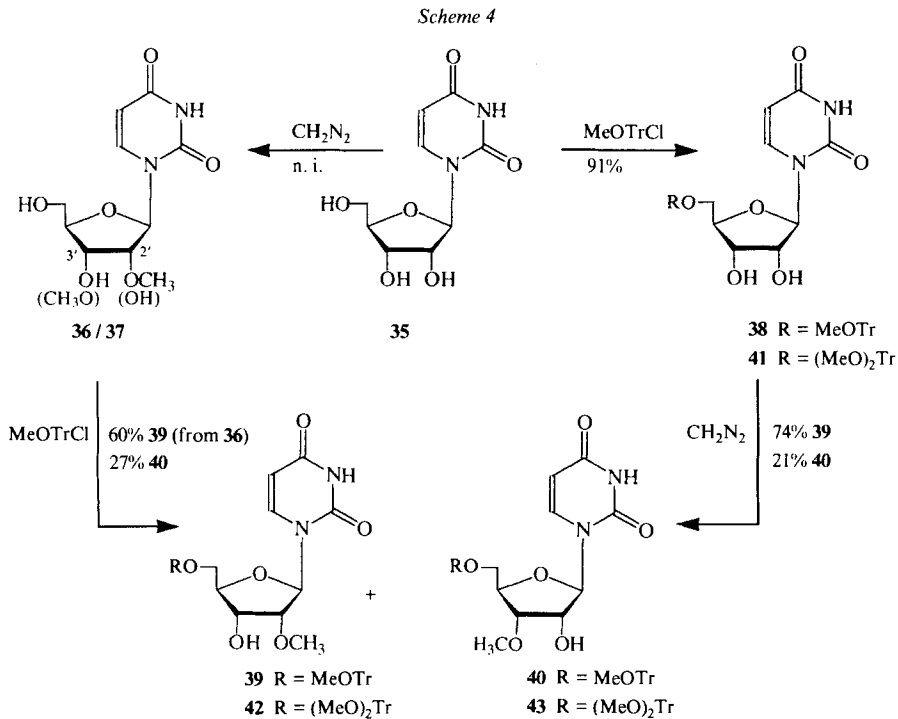
<sup>c)</sup> The corresponding 3'-O-methyl isomer 29 was also obtained in 5% yield from 34.

Table 5. 2':3' Ratio for the Methylation of 5'-O-(Monomethoxytrityl)-N<sup>4</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]-cytidine (**34**) under Different Reaction Conditions

Scale [mmol]	Solvent [ml]	CH <sub>2</sub> N <sub>2</sub> Solution			Ratio 2':3' ( <b>28/29</b> ) <sup>c</sup>	% N- methylation	T [°C]
		Vol [ml]	N <sup>a</sup>	t [min] <sup>b</sup>			
0.5	DMF	1	0.73	0.25	87:13	10	0
0.5	DMF	1.3	0.65	0.75	87:13	15	-40 <sup>d</sup>
0.5	DMA	1.3	0.65	0.75	86:14	5	0
5.0	DMA	35	0.15	30	86:14	1	20
5.0	DMA	35	0.15	20	88:12	–	0
1.0	CH <sub>2</sub> Cl <sub>2</sub>	20	0.08	25	93:07	2	0
5.0	CH <sub>2</sub> Cl <sub>2</sub>	18	0.5	5	92:08	1.5	0
5.0	CH <sub>2</sub> Cl <sub>2</sub>	20	0.45	10	93:07	2	20
1.0	CH <sub>2</sub> Cl <sub>2</sub> /(2% Et <sub>3</sub> N)	15	0.1	20	89:11	–	20
1.0	CHCl <sub>3</sub>	17	0.1	30	88:12	3	20
1.0	dioxane	15	0.1	30	90:10	–	20
1.0	TMU	28	0.1	15	86:14	–	20
1.0	toluene	15	0.1	30	78:22	10	20
1.0	AcOEt	25	0.08	30	91:09	8	0
1.0	acetone	20	0.08	30	79:21	5	0
1.0	MeCN	19	0.08	30	81:19	6	0

<sup>a</sup>)<sup>b</sup>)<sup>c</sup>) See Table 2. <sup>d</sup>) Slowly warming up within 3 h to -20°.

2'- and 3'-O-Methyluridine Derivatives. Robins and coworkers [42] were able to isolate 58% of 2'-O-methyl isomer **36** and 28% of 3'-O-methyl isomer **37** after the methylation of uridine (**35**) was performed with diazomethane (Scheme 4). To avoid base methylation,



the diazomethane solution had to be added much more slowly than in the case of adenosine (**9**) or cytidine (**23**), and separation of the isomers was best accomplished by prep. TLC.

Our attempts failed to find a solvent system for the separation of **36** and **37** by column chromatography (silica gel). Thus, we decided to introduce the trityl group *prior* to methylation (**35** → **38** → **39/40**; *Scheme 4*). The tritylated products could then easily be separated by FC (silica gel) to give 2'-*O*-methyl-5'-*O*-(monomethoxytrityl)uridine (**39**) and its 3'-*O*-methyl isomer **40** in 60 and 27%, respectively.

We studied also the influence of different solvents and the scale size on the 2':3' ratio when methylating 5'-*O*-(monomethoxytrityl)uridine (**38**), but surprisingly a change of these parameters decreased allways the amount of the 2'-isomer **39** formed. With some solvents, a considerable amount of *N*-methylation took place (see *Table 6*), especially when adding the diazomethane solution too fast. In AcOEt or MeCN, partial cleavage of the 5'-*O*-monomethoxytrityl protecting group occurred during methylation. Dioxane is recommended as the solvent of choice though besides 74% of **39** also 21% of **40** was formed. To invert the 2':3' ratio, THF should be chosen as solvent.

The methylation of 5'-*O*-(dimethoxytrityl)uridine (**41**) gave 2'-*O*-methyl-5'-*O*-(dimethoxytrityl)uridine (**42**) and its 3'-*O*-methyl isomer **43** in 71 and 22% yield, respectively. If desired, detritylation of **39** and **40** leads to 2'- and 3'-*O*-methyluridine (**36** and **37**, resp.) in *ca.* 85% yields.

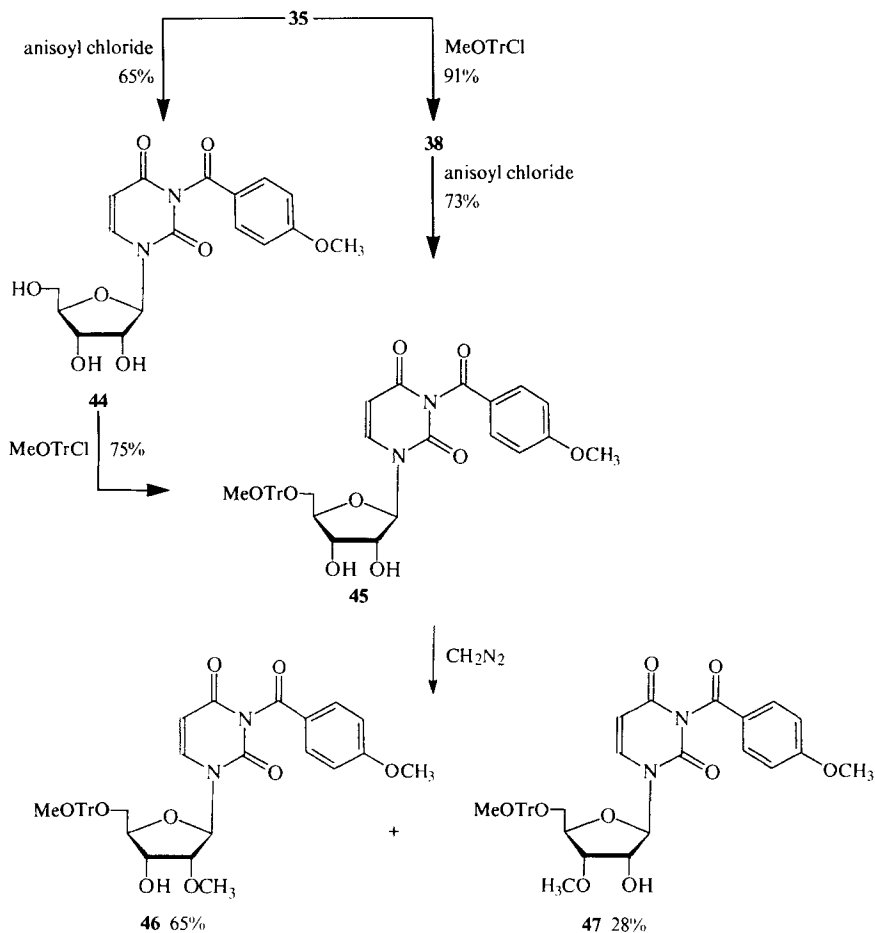
Table 6. 2':3' Ratio for the Methylation of 5'-*O*-(Monomethoxytrityl)uridine (**38**) under Different Reaction Conditions (*T* 20°, if not noted otherwise)

Scale [mmol]	Solvent [ml]	CH <sub>2</sub> N <sub>2</sub> Solution			Ratio 2':3' ( <b>39/40</b> ) <sup>c</sup>	% <i>N</i> - methylation	SnCl <sub>2</sub> [equiv.]
		Vol [ml]	<i>N</i> <sup>a)</sup>	<i>t</i> [min] <sup>b)</sup>			
1.0	CH <sub>2</sub> Cl <sub>2</sub> (50)	20	0.08	20	52:48	–	0.05
10.0	CH <sub>2</sub> Cl <sub>2</sub> (500)	40	0.4	60	48:52	–	0.025
10.0	CH <sub>2</sub> Cl <sub>2</sub> (500)	40	0.4	30	45:55	–	0.025
20.0	CH <sub>2</sub> Cl <sub>2</sub> (500)	80	0.4	30	43:57	–	0.01
1.0	CH <sub>2</sub> Cl <sub>2</sub> /(1% Et <sub>3</sub> N)	20	0.1	90	64:36	17	0.05
1.0	CHCl <sub>3</sub> (50)	20	0.1	10	54:46	2	0.05
1.0	dioxane (50)	25	0.1	60	73:27	1	0.05
5.0 <sup>d)</sup>	dioxane (125)	10	1.0	10	76:24	1	0.025
10.0	dioxane (250)	50	0.4	15	71:29	1	0.025
10.0	dioxane (250)	20	1.0	10	78:22	1	0.025
1.0	TMU (25)	20	0.08	20	73:27	4	0.05
10.0	TMU (250)	40	0.5	60	73:27	–	0.10
10.0	TMU (250)/0°	40	0.5	30	71:29	–	0.10
20.0	TMU (125)	35	1.0	10	65:35	3	0.10
1.0	DMF (30)	20	0.08	30	70:30	7	0.05
5.0	DMF (150)	50	0.2	10	62:38	12	0.05
1.0	DMA (30)	20	0.08	30	69:31	10	0.05
1.0	acetone (50)	40	0.08	30	58:42	–	0.05
1.0	THF (50)	20	0.08	20	41:59	–	0.05
1.0	pyridine (50)	20	0.08	60	67:33	15	0.05
1.0	toluene (50)	20	0.1	30	66:34	5	0.05
1.0	AcOEt (50)	50	0.08	30	55:45	15% detrit.	0.05
1.0	MeCN (50)	30	0.08	30	59:41	30% detrit.	0.05

<sup>a)</sup> <sup>b)</sup> <sup>c)</sup> See *Table 2*. <sup>d)</sup> 5'-*O*-(Dimethoxytrityl)uridine (**41**) was used as starting material. <sup>e)</sup> The solution turned deep red when adding diazomethane solution.

$N^3$ -Anisoyl-protected uridine derivatives were synthesized as well (Scheme 5). Methylation was carried out on  $N^3$ -anisoyl-5'- $O$ -(monomethoxytrityl)uridine (**45**) which can be synthesized from uridine (**35**) either *via*  $N^3$ -anisoyluridine (**44**) [65] or 5'- $O$ -(monomethoxytrityl)uridine (**38**) [66] (see also Scheme 4).  $N^3$ -Anisoyl-2'- $O$ -methyl-5'- $O$ -(monomethoxytrityl)uridine (**46**) and its 3'- $O$ -methyl isomer **47** could be isolated after methylation of **45** in dioxane and FC (silica gel) in 65 and 28% yield, respectively.

Scheme 5



**3. Conclusion.** – With the herein reported methods for the  $O$ -methylation of the four nucleosides guanosine, adenosine, cytidine, and uridine and its derivatives, an increase of the amount of the 2'- $O$ -methyl isomer could be achieved in comparison with previously reported methods. The methylation results of *Robins* and coworkers with all four bases using diazomethane and the catalyst stannous chloride could be significantly improved

by applying this method to partially protected ribonucleosides. The introduction of the (eventually needed) protecting groups for the oligonucleotide synthesis *prior* to methylation significantly increased the amount of 2'-*O*-methyl isomers formed, from 40 to 79% for guanosine, from 35 to 63% for adenosine, from 74 to 87% for cytidine, and from 58 to 74% for uridine (yields after isolation). Concomitantly, the protecting groups made it possible to separate the 2':3'-isomer mixture more easily by FC (silica gel).

The potentially hazardous diazomethane which was used for the methylation was prepared from *N*-methyl-*N*-nitrosourea in alkaline 1,2-dimethoxyethane solution, thus being directly ready to use without distillation. Methylation of the protected rather than the unprotected nucleosides was also accompanied by a reduction of the excess of diazomethane solution. For the methylation of the MeOTr-protected nucleosides, normally 1.5 equiv. of diazomethane was sufficient for a complete reaction, whereas the unprotected nucleosides uridine, cytidine, and adenosine in MeOH needed a 15, 4, and 11 equiv. excess, respectively.

**4. Physical Data.** – All new synthesized compounds were characterized by elemental analysis and UV and <sup>1</sup>H-NMR spectra. The methyl group does not have a chromophor, and thus the 2'- and 3'-*O*-methyl derivatives show the same UV spectra as the unmethylated nucleosides [61].

Assignments of the 2'- and 3'-*O*-methyl isomer can be based on <sup>1</sup>H-NMR spectra since the signal for the 2'-*O*-methyl group is usually found downfield from the 3'-*O*-methyl signal in the region 3.3–3.7 ppm. The only exceptions so far are 2'- and 3'-*O*-methyladenosine (**10** and **11**, resp.) and 2'- and 3'-*O*-methyl-*N*<sup>6</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**12** and **13**, resp.) showing a reverse chemical-shift behavior. The anomeric proton H–C(1') is best suitable for the determination of the 2':3' ratio in an isomeric mixture because it does not overlap with other signals of the molecule. Furthermore, the H–C(1') signal of the 2'-*O*-methyl isomer is always at lower field compared with the 3'-*O*-methyl isomer.

The introduction of a Me group causes also a change in the sequence of the chemical shifts of the sugar protons which are orientated from low field (H–C(1')) to high field (H–C(5')) in unsubstituted nucleosides. The presence of the electron-donating Me group in the 2'-*O*-position is in general associated with an upfield shift of the adjacent H–C proton, thus allowing another specific structure determination.

#### Experimental Part

*General.* Products were dried under high vacuum. TLC: precoated silica gel thin-layer sheets *F1500 LS 254* from *Schleicher & Schuell* or *60 F<sub>254</sub>* from *Merck*. Flash chromatography (FC): silica gel (*Baker*, 30–60 μm); 0.2–0.3 bar. M.p.: *Gallenkamp* or *Büchi-510* melting-point apparatus; no corrections. UV/VIS: *Perkin-Elmer, Lambda 15*; λ<sub>max</sub> in nm (log ε). <sup>1</sup>H-NMR: *Bruker AC 250*: in ppm rel. to SiMe<sub>4</sub> or CDCl<sub>3</sub> ((D<sub>6</sub>)DMSO, D<sub>2</sub>O) as internal standard. Abbreviations: DME, 1,2-dimethoxyethane; DMA, *N,N*-dimethylacetamide; TMU, *N,N,N',N'*-tetramethylurea; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene.

*Diazomethane Solution.* To DME (150 ml) and 40% aq. KOH (100 ml) at 0°, *N*-methyl-*N*-nitrosourea (30 g) was added slowly with vigorous stirring so that the temp. did not exceed 5°. Stirring was continued for an additional 20 min at 0°. The phases were allowed to separate. The upper org. layer was decanted, the aqueous phase washed twice with DME (25 ml), and the combined org. phase dried at 0° over KOH pellets. The DME soln. was decanted from the KOH, the KOH washed twice with DME (10 ml) and filtered, if needed. This yielded 170 ml of a 1.0*N* CH<sub>2</sub>N<sub>2</sub> soln. in DME which was used directly for the methylation (standardization of the soln. was achieved

by adding a defined amount of 0.2M benzoic acid in DME and back titration with 0.1M NaOH using the indicator phenolphthalein). For the methylation on smaller scales, the  $\text{CH}_2\text{N}_2$  soln. was diluted prior to use.

2'-O-Methyl-5'-O-(monomethoxytrityl)-N<sup>2</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]-O<sup>6</sup>-[2-(4-nitrophenyl)ethyl]guanosine (3) and 3'-O-Methyl-5'-O-(monomethoxytrityl)-N<sup>2</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]-O<sup>6</sup>-[2-(4-nitrophenyl)ethyl]guanosine (4). A soln. of 5'-O-(monomethoxytrityl)-N<sup>2</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]-O<sup>6</sup>-[2-(4-nitrophenyl)ethyl]guanosine (1) [60] (8.98 g, 10.0 mmol) and  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  (100 mg, 0.44 mmol) in DMA (250 ml) was cooled to  $-10^\circ$  (salt/ice-bath), and then a freshly prepared  $\text{CH}_2\text{N}_2$  soln. in DME (20 ml, 0.84M) was added dropwise within 45 min (TLC (toluene/acetone 2:1): complete conversion). The mixture was evaporated, dried under high vacuum, co-evaporated with  $\text{CH}_2\text{Cl}_2$ , and again dried under high vacuum. <sup>1</sup>H-NMR: 3/4 83:17. The product mixture was taken up in  $\text{CH}_2\text{Cl}_2$  and separated by FC (silica gel, 600 g, 25 × 8.5 cm, petroleum ether/ $\text{CHCl}_3$ /EtOH 100:50:0 (packed), 100:50:5 (6.0); 4), 100:50:6 (2.3); 4 and then 3), 100:50:8 (2.3 1), and 100:50:10 (1.5 1)): 1.19 g (13%) of 4, 275 mg (3%) of 3/4, and 7.13 g (78%) of 3 as colorless foams. Separation of mixed fractions by FC (15 g, same solvent system) yielded 176 mg (2%) of 4 and 89 mg (1%) of 3. Combined yield: 7.22 g (79%) of 3 and 1.27 g (15%) of 4.

3: TLC ( $\text{SiO}_2$ , petroleum ether/ $\text{CHCl}_3$ /EtOH 10:5:1):  $R_f$  0.37. UV (MeOH): 269 (4.54), 235 (4.37), 205 (4.88). <sup>1</sup>H-NMR ( $\text{CDCl}_3$ ): 8.16 (d, 4 H o to  $\text{NO}_2$ ); 8.01 (s, H-C(8)); 7.53-7.16 (m, 16 H, MeOTr, 4 H m to  $\text{NO}_2$ ); 6.79 (d, 2 H o to MeO); 6.07 (d, H-C(1')); 4.81 (t,  $\text{CH}_2\text{O}$ ); 4.59 (q, H-C(3')); 4.43 (m,  $\text{CH}_2\text{OCO}$ , H-C(2')); 4.17 (m, H-C(4')); 3.77 (s, MeOTr); 3.58 (s, MeO-C(2')); 3.49 (dd, 1 H-C(5')); 3.43 (dd, 1 H-C(5')); 3.31 (t,  $\text{ArCH}_2$ ); 3.08 (t,  $\text{ArCH}_2$ ); 2.62 (d, OH-C(3')). Anal. calc. for  $\text{C}_{48}\text{H}_{45}\text{N}_7\text{O}_{12}$  (911.9): C 63.22, H 4.97, N 10.75; found: C 62.67, H 5.02, N 10.44.

4: TLC ( $\text{SiO}_2$ , petroleum ether/ $\text{CHCl}_3$ /EtOH 10:5:1):  $R_f$  0.45. UV (MeOH): 269 (4.53), 235 (4.37), 205 (4.88). <sup>1</sup>H-NMR ( $\text{CDCl}_3$ ): 8.14 (t, 4 H o to  $\text{NO}_2$ ); 8.05 (s, H-C(8)); 7.48-7.11 (m, 16 H, MeOTr, 4 H m to  $\text{NO}_2$ ); 6.74 (d, 2 H o to MeO); 5.89 (d, H-C(1')); 5.79 (d, OH-C(2')); 4.97 (q, H-C(2')); 4.76 (t,  $\text{CH}_2\text{O}$ ); 4.47 (m,  $\text{CH}_2\text{OCO}$ ); 4.37 (m, H-C(4')); 4.10 (d, H-C(3')); 3.75 (s, MeOTr); 3.54 (s, MeO-C(3')); 3.34 (dd, 1 H-C(5')); 3.28 (t,  $\text{ArCH}_2$ ); 3.20 (dd, 1 H-C(5')); 3.10 (t,  $\text{ArCH}_2$ ). Anal. calc. for  $\text{C}_{48}\text{H}_{45}\text{N}_7\text{O}_{12} \cdot \text{H}_2\text{O}$  (926.0): C 62.26, H 5.12, N 10.59; found: C 61.61, H 5.22, N 10.29.

5'-O-(Dimethoxytrityl)-2'-O-methyl-N<sup>2</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]-O<sup>6</sup>-[2-(4-nitrophenyl)ethyl]guanosine (5) and 5'-O-(Dimethoxytrityl)-3'-O-methyl-N<sup>2</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]-O<sup>6</sup>-[2-(4-nitrophenyl)ethyl]guanosine (6). A soln. of 5'-O-(dimethoxytrityl)-N<sup>2</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]-O<sup>6</sup>-[2-(4-nitrophenyl)ethyl]guanosine (2) [60] (1.28 g, 1.38 mmol) and  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  (15 mg, 0.066 mmol) in DMA (35 ml) was cooled to  $-10^\circ$  (salt/ice-bath). Then a freshly prepared  $\text{CH}_2\text{N}_2$  soln. in DME (12 ml, 0.4M) was added dropwise within 10 min (TLC (toluene/acetone 2:1): complete conversion). Workup as described for 3/4. <sup>1</sup>H-NMR: 5/6 81:19. FC (silica gel, 75 g, 17 × 4 cm, petroleum ether/ $\text{CHCl}_3$ /EtOH 100:50:0 (packed), 100:50:5 (2000 ml, 6 and then 5) and 100:50:8 (300 ml)): 209 mg (16%) of 6 and 956 mg (76%) of 5. Colorless foams.

5: TLC ( $\text{SiO}_2$ , petroleum ether/ $\text{CHCl}_3$ /EtOH 10:5:1):  $R_f$  0.37. UV (MeOH): 269 (4.57), 236 (4.49), 204 (4.94). <sup>1</sup>H-NMR ( $\text{CDCl}_3$ ): 8.14 (d, 4 H o to  $\text{NO}_2$ ); 8.02 (s, H-C(8)); 7.53-7.11 (m, 13 H, (MeO)<sub>2</sub>Tr, 4 H m to  $\text{NO}_2$ ); 6.78 (d, 4 H o to MeO); 6.07 (d, H-C(1')); 4.80 (t,  $\text{CH}_2\text{O}$ ); 4.60 (m, H-C(3')); 4.42 (m,  $\text{CH}_2\text{OCO}$ , H-C(2')); 4.16 (m, H-C(4')); 3.76 (s, (MeO)<sub>2</sub>Tr); 3.57 (s, MeO-C(2')); 3.48 (dd, 1 H-C(5')); 3.41 (dd, 1 H-C(5')); 3.30 (t,  $\text{ArCH}_2$ ); 3.07 (t,  $\text{ArCH}_2$ ); 2.72 (d, OH-C(3')). Anal. calc. for  $\text{C}_{49}\text{H}_{47}\text{N}_7\text{O}_{13}$  (941.9): C 62.48, H 5.03, N 10.41; found: C 61.96, H 5.18, N 10.18.

6: TLC ( $\text{SiO}_2$ , petroleum ether/ $\text{CHCl}_3$ /EtOH 10:5:1):  $R_f$  0.45. UV (MeOH): 269 (4.55), 236 (4.48), 204 (4.93). <sup>1</sup>H-NMR ( $\text{CDCl}_3$ ): 8.16 (t, 4 H o to  $\text{NO}_2$ ); 8.05 (s, H-C(8)); 7.48-7.15 (m, 13 H, (MeO)<sub>2</sub>Tr, 4 H m to  $\text{NO}_2$ ); 6.72 (d, 4 H o to MeO); 5.89 (d, H-C(1')); 5.80 (d, OH-C(2')); 4.97 (q, H-C(2')); 4.77 (t,  $\text{CH}_2\text{O}$ ); 4.47 (m,  $\text{CH}_2\text{OCO}$ ); 4.37 (m, H-C(4')); 4.10 (d, H-C(3')); 3.75 (s, (MeO)<sub>2</sub>Tr); 3.55 (s, MeO-C(3')); 3.32 (dd, 1 H-C(5')); 3.29 (t,  $\text{ArCH}_2$ ); 3.19 (dd, 1 H-C(5')); 3.11 (t,  $\text{ArCH}_2$ ). Anal. calc. for  $\text{C}_{49}\text{H}_{47}\text{N}_7\text{O}_{13}$  (941.9): C 62.48, H 5.03, N 10.41; found: C 61.95, H 5.24, N 10.24.

2'-O-Methyl-5'-O-(monomethoxytrityl)guanosine (7). A mixture of 3 (912 mg, 1.00 mmol) and 1M DBU in MeCN (20 ml) was stirred at r.t. After 9 h, the mixture was neutralized with AcOH (1.2 ml) and concentrated to ¼ of the original volume. This concentrated mixture was added dropwise to ice-cold  $\text{H}_2\text{O}$  (150 ml). The precipitate was collected, washed with ice-cold  $\text{H}_2\text{O}$  and dried under high vacuum. Crystallization from acetone (20 ml) gave 443 mg (78%) of 7. Colorless needles. M.p. 208° (acetone). TLC ( $\text{SiO}_2$ ,  $\text{CH}_2\text{Cl}_2$ /MeOH 9:1):  $R_f$  0.31. UV (MeOH): 263 (sh, 4.14), 248 (4.24), 234 (4.29), 205 (4.74). <sup>1</sup>H-NMR ((D<sub>6</sub>)DMSO): 10.65 (s, NH); 7.81 (s, H-C(8)); 7.38-7.20 (m, 12 H, MeOTr); 6.79 (d, 2 H o to MeO); 6.47 (s,  $\text{NH}_2$ ); 5.83 (d, H-C(1')); 5.24 (d, OH-C(3')); 4.28 (q, H-C(3')); 4.19 (t, H-C(2')); 3.99 (m, H-C(4')); 3.73 (s, MeOTr); 3.36 (s, MeO-C(2')); 3.17 (m, 2 H-C(5')). Anal. calc. for  $\text{C}_{31}\text{H}_{31}\text{N}_5\text{O}_6$  (569.6): C 65.37, H 5.49, N 12.30; found: C 64.94, H 5.66, N 11.87.



**2'-O-Methylguanosine (8).** A mixture of **7** (285 mg, 0.50 mmol) and 2% TsOH in CH<sub>2</sub>Cl<sub>2</sub>/MeOH 4:1 (5 ml) was stirred at r.t. for 15 min, then neutralized with some drops of NaOMe soln., and evaporated. The residue was taken up in CH<sub>2</sub>Cl<sub>2</sub>/MeOH 10:1 and purified by FC (silica gel, 10 g, 11 × 2 cm, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 20:1 (100 ml), 9:1 (100 ml), 7:1 (100 ml), 5:1 (100 ml), and 4:1 (100 ml)): 149 mg of **8** [42] (containing ca. 10% of TsOH). The crude product was crystallized from H<sub>2</sub>O (5 ml): 89 mg (60%). Colorless fine crystals. M.p. 225–226° (H<sub>2</sub>O). TLC (SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 2:1): R<sub>f</sub> 0.63. UV (MeOH): 266 (sh, 4.03), 253 (4.16). <sup>1</sup>H-NMR ((D<sub>6</sub>)DMSO): 10.63 (s, NH); 7.96 (s, H-C(8)); 6.46 (s, NH<sub>2</sub>); 5.78 (d, H-C(1')); 5.20 (d, OH-C(3')); 5.08 (t, OH-C(5')); 4.25 (q, H-C(3')); 4.15 (t, H-C(2')); 3.88 (q, H-C(4')); 3.55 (m, 2 H-C(5')); 3.30 (s, MeO-C(2')). Anal. calc. for C<sub>11</sub>H<sub>15</sub>N<sub>5</sub>O<sub>5</sub> · ½ H<sub>2</sub>O (306.3): C 43.13, H 5.27, N 22.87; found: C 43.04, H 5.41, N 22.61.

**2'-O-Methyl-N<sup>6</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (12).** A mixture of dry 2'-O-methyladenosine (**10**) [42] (1.13 g, 4.0 mmol), hexamethyldisilazane (7.5 ml, 5.8 g, 36 mmol), and a catal. amount of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was heated under reflux in anh. dioxane (10 ml) for 3.5 h (TLC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1): complete conversion of **10** (R<sub>f</sub> 0.20) into the persilylated product (R<sub>f</sub> 0.60)). After cooling, the mixture was evaporated and twice co-evaporated with anh. toluene. The residue was dissolved in anh. CH<sub>2</sub>Cl<sub>2</sub> (30 ml), 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1*H*-imidazol-3-ium chloride [63] (1.62 g, 5.20 mmol) was added, and the mixture stirred at r.t. If TLC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 20:1) did not show complete reaction after 20 h, more acylating agent was added and the mixture stirred until completion. Otherwise undissolved acylating agent was filtered off, if needed. The solvent was evaporated and the residue taken up in MeOH (20 ml) and, for the hydrolysis of the Me<sub>3</sub>Si groups, stirred together with Et<sub>3</sub>N (5 ml) overnight at r.t. The soln. was evaporated and purified by FC (silica gel, 60 g, 11 × 4 cm, CH<sub>2</sub>Cl<sub>2</sub> (500 ml), CH<sub>2</sub>Cl<sub>2</sub>/MeOH 100:1 (200 ml), 100:3 (200 ml), 100:5 (300 ml), and 100:7 (400 ml)) to give 1.62 g (85%) of **12**. Colorless amorphous solid. M.p. 145° (MeOH). TLC (SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 20:1): R<sub>f</sub> 0.39. UV (MeOH): 267 (4.40), 208 (4.48). <sup>1</sup>H-NMR ((D<sub>6</sub>)DMSO): 10.63 (s, NH); 8.71 (s, H-C(8)); 8.62 (s, H-C(2)); 8.15 (d, 2 H *o* to NO<sub>2</sub>); 7.60 (d, 2 H *m* to NO<sub>2</sub>); 6.11 (d, H-C(1')); 5.33 (d, OH-C(3')); 5.19 (t, OH-C(5')); 4.39 (m, H-C(2'), H-C(3'), CH<sub>2</sub>O); 3.98 (m, H-C(4')); 3.64 (m, 2 H-C(5')); 3.33 (s, MeO-C(2')); 3.10 (t, ArCH<sub>2</sub>). Anal. calc. for C<sub>20</sub>H<sub>22</sub>N<sub>6</sub>O<sub>8</sub> · ½ H<sub>2</sub>O (492.4): C 49.69, H 4.80, N 17.38; found: C 49.34, H 4.95, N 17.22.

**3'-O-Methyl-N<sup>6</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (13).** As described for **12**, with *O*-methyladenosine (**11**) [42] (281 mg, 1.0 mmol), hexamethyldisilazane (2.3 ml, 1.78 g, 11 mmol), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, dioxane (2.5 ml) (for 3 h; TLC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1): complete conversion; R<sub>f</sub> 0.20 (**11**), 0.67 (persilylated product)), then CH<sub>2</sub>Cl<sub>2</sub> (10 ml), and 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1*H*-imidazol-3-ium chloride (410 mg, 1.30 mmol). The residue was stirred in MeOH (10 ml) and Et<sub>3</sub>N (2.5 ml) overnight at r.t. whereby **13** crystallized. The precipitate was filtered by suction, washed with MeOH and Et<sub>2</sub>O, and dried at 40°/high vacuum: 451 mg (95%) of **13**. TLC (SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 20:1): R<sub>f</sub> 0.35. Colorless powder. M.p. 169° (dioxane). UV (MeOH): 266 (4.43), 210 (4.50). <sup>1</sup>H-NMR ((D<sub>6</sub>)DMSO): 10.61 (s, NH); 8.67 (s, H-C(8)); 8.62 (s, H-C(2)); 8.16 (d, 2 H *o* to NO<sub>2</sub>); 7.61 (d, 2 H *m* to NO<sub>2</sub>); 5.98 (d, H-C(1')); 5.59 (d, OH-C(2')); 5.19 (t, OH-C(5')); 4.77 (q, H-C(2')); 4.38 (t, CH<sub>2</sub>O); 4.07 (m, H-C(4')); 3.89 (t, H-C(3')); 3.68 (m, 1 H-C(5')); 3.58 (m, 1 H-C(5')); 3.40 (s, MeO-C(3')); 3.11 (t, ArCH<sub>2</sub>). Anal. calc. for C<sub>20</sub>H<sub>22</sub>N<sub>6</sub>O<sub>8</sub> (474.4): C 50.63, H 4.67, N 17.71; found: C 50.33, H 4.87, N 17.48.

**2'-O-Methyl-5'-O-(monomethoxytrityl)-N<sup>6</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (14).** a) Compound **12** (1.37 g, 2.72 mmol) was co-evaporated twice with dry pyridine and then dissolved in dry pyridine (10 ml). MeOTrCl (510 mg, 1.5 mmol) was added and the soln. stirred for 72 h at r.t. The mixture was concentrated, diluted with CH<sub>2</sub>Cl<sub>2</sub> (50 ml), and washed with phosphate buffer (pH 7, 50 ml). The aq. phase was extracted twice with CH<sub>2</sub>Cl<sub>2</sub> (25 ml) and the combined org. phase washed with phosphate buffer (pH 7, 25 ml). The org. phase was dried (Na<sub>2</sub>SO<sub>4</sub>), evaporated, and co-evaporated 3× with toluene (10 ml). The crude product was purified by FC (silica gel, 50 g, 12 × 3.5 cm, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 100:1 (700 ml), 100:2 (500 ml), and 100:3 (200 ml), or toluene/acetone 3:1 (500 ml), 2:1 (200 ml), and 1:1 (200 ml)): 1.68 g (83%) of **14**. Colorless foam.

b) As described for **12**, with **21** (6.46 g, 11.6 mmol), hexamethyldisilazane (14.5 ml, 11.3 g, 70 mmol), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, dioxane (40 ml) (3 h; TLC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 100:2): complete conversion), then CH<sub>2</sub>Cl<sub>2</sub> (80 ml), and 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1*H*-imidazol-3-ium chloride (4.36 g, 14.0 mmol). The residue was taken up in acetone (20 ml) and stirred together with MeOH (20 ml) and Et<sub>3</sub>N (10 ml) overnight at r.t. The soln. was evaporated and the residue purified by FC (silica gel, 200 g, 19 × 5.5 cm, toluene/acetone 4:1 (1 l), 7:2 (3 l, **14**), 3:1 (1 l, **14**), and 2:1 (500 ml, impurities of **15** if any)): 7.84 g (91%) of **14**. Colorless foam. TLC (SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 20:1 or toluene/acetone 2:1): 0.60 and 0.38, resp. UV (MeOH): 267 (4.46), 232 (4.29), 205 (4.78). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 8.69 (s, H-C(8)); 8.39 (br., NH); 8.21 (s, H-C(2)); 8.17 (d, 2 H *o* to NO<sub>2</sub>); 7.44–7.18 (m, 14 H, MeOTr, 2 H *m* to NO<sub>2</sub>); 6.81 (d, 2 H *o* to MeO); 6.18 (d, H-C(1')); 4.53 (t, H-C(3'), CH<sub>2</sub>O); 4.44 (t, H-C(2')); 4.23 (q, H-C(4')); 3.78 (s, MeOTr); 3.57 (s, MeO-C(2')); 3.54 (dd, 1 H-C(5')); 3.43 (dd, 1 H-C(5')); 3.14 (t, ArCH<sub>2</sub>); 2.80 (d, OH-C(3')). Anal. calc. for C<sub>40</sub>H<sub>38</sub>N<sub>6</sub>O<sub>9</sub> (746.8): C 64.33, H 5.13, N 11.25; found: C 64.16, H 5.39, N 10.62.

**3'-O-Methyl-5'-O-(monomethoxytrityl)-N<sup>6</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (15).** a) As described for **14** (see a)), with **13** (366 mg, 0.77 mmol), pyridine (3 ml), and MeOTrCl (350 mg, 1.16 mmol). Workup with CH<sub>2</sub>Cl<sub>2</sub> (20 ml), phosphate buffer (pH 7, 20 ml), CH<sub>2</sub>Cl<sub>2</sub> (10 ml), and phosphate buffer (pH 7, 10 ml). FC (silica gel, 15 g, 11 × 2.5 cm, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 100:1 (100 ml), 100:2 (200 ml), and 100:3 (100 ml), or toluene/acetone 2:1 (100 ml), 1:1 (200 ml), and 1:2 (100 ml)) gave 485 mg (85%) of **15**. Colorless foam.

b) As described for **12**, with **22** (3.80 g, 6.86 mmol), hexamethyldisilazane (8.5 ml, 6.6 g, 41 mmol), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>-dioxane (20 ml) (3 h; TLC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 100:2): complete conversion), then CH<sub>2</sub>Cl<sub>2</sub> (50 ml), and 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1*H*-imidazol-3-ium chloride (2.59 g, 8.3 mmol). The residue was taken up in acetone (20 ml) and stirred together with MeOH (20 ml) and Et<sub>3</sub>N (10 ml) overnight at r.t. The soln. was evaporated and purified by FC (silica gel, 200 g, 19 × 5.5 cm, toluene/acetone 4:1 (1 l), 7:2 (2 l, impurities of **14** if any), 3:1 (0.5 l, **15**), 2:1 (1.2 l, **15**), and 1:1 (0.5 l, **15**)): 4.24 g (83%) of **15**. Colorless foam. TLC (SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 20:1 or toluene/acetone 2:1): 0.54 and 0.30, resp. UV (MeOH): 266 (4.48), 232 (4.32), 205 (4.81). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 8.68 (s, H-C(8)); 8.49 (s, NH); 8.16 (d, 2 H o to NO<sub>2</sub>); 8.15 (s, H-C(2)); 7.42–7.18 (m, 14 H, MeOTr, 2 H m to NO<sub>2</sub>); 6.80 (d, 2 H o to MeO); 6.00 (d, H-C(1')); 4.92 (q, H-C(2')); 4.52 (t, CH<sub>2</sub>O); 4.32 (q, H-C(4')); 4.10 (t, H-C(3')); 3.77 (s, MeOTr, OH-C(2')); 3.48 (s, MeO-C(3')); 3.49 (dd, 1 H-C(5')); 3.33 (dd, 1 H-C(5')); 3.13 (t, ArCH<sub>2</sub>). Anal. calc. for C<sub>40</sub>H<sub>38</sub>N<sub>6</sub>O<sub>9</sub> (746.8): C 64.33, H 5.13, N 11.25; found: C 64.26, H 5.49, N 10.77.

**5'-O-(Dimethoxytrityl)-2'-O-methyl-N<sup>6</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (16).** As described for **14** (see a)), with **12** (711 mg, 1.5 mmol), pyridine (5 ml), and (MeO)<sub>2</sub>TrCl (510 mg, 1.5 mmol). Workup with CH<sub>2</sub>Cl<sub>2</sub> (30 ml), phosphate buffer (pH 7, 30 ml), CH<sub>2</sub>Cl<sub>2</sub> (15 ml), and phosphate buffer (pH 7, 15 ml). FC (silica gel, 28 g, 16 × 2.5 cm, toluene/acetone 3:1 (200 ml), 2:1 (100 ml), and 1:1 (100 ml)) gave 916 mg (79%) of **16**. Colorless foam. TLC (SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 20:1): R<sub>f</sub> 0.60. UV (MeOH): 267 (4.47), 235 (4.44), 205 (4.90). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 8.70 (s, H-C(8)); 8.49 (s, NH); 8.22 (s, H-C(2)); 8.16 (d, 2 H o to NO<sub>2</sub>); 7.44–7.17 (m, 11 H, (MeO)<sub>2</sub>Tr, 2 H m to NO<sub>2</sub>); 6.80 (d, 4 H o to MeO); 6.19 (d, H-C(1')); 4.52 (t, H-C(3'), CH<sub>2</sub>O); 4.43 (t, H-C(2')); 4.22 (q, H-C(4')); 3.77 (s, (MeO)<sub>2</sub>Tr); 3.56 (s, MeO-C(2')); 3.55 (dd, H-C(5')); 3.46 (dd, H-C(5')); 3.13 (t, ArCH<sub>2</sub>); 2.87 (d, OH-C(3')). Anal. calc. for C<sub>41</sub>H<sub>40</sub>N<sub>6</sub>O<sub>10</sub> · H<sub>2</sub>O (794.8): C 61.95, H 5.32, N 10.22; found: C 62.12, H 5.18, N 10.22.

**2'-O-Methyl-5'-O-(monomethoxytrityl)adenosine (21) and 3'-O-Methyl-5'-O-(monomethoxytrityl)adenosine (22).** To a soln. of 5'-O-(monomethoxytrityl)adenosine (**20**) [67] [68] (8.09 g, 15.0 mmol) and SnCl<sub>2</sub> · 2 H<sub>2</sub>O (225 mg, 1.0 mmol) in DMSO (250 ml), freshly prepared 0.8M CH<sub>2</sub>N<sub>2</sub> soln. (32 ml) was added dropwise at r.t. within 35 min (TLC: complete conversion). The solvent was evaporated and the residue dried under high vacuum, co-evaporated with CH<sub>2</sub>Cl<sub>2</sub>, and again dried under high vacuum. <sup>1</sup>H-NMR: **21/22** 67:33. Separation of the isomers was achieved by FC (silica gel, 400 g, 28 × 6.5 cm, toluene/acetone 2:1 (2 l), 3:2 (4 l, **21**, then **22**), 1:1 (1 l, **22**), 1:2 (0.5 l), and 1:4 (1.5 l)): 5.25 (63%) of **21** [69] and 2.56 (31%) of **22** [69]. Colorless foams.

**21:** TLC (SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 20:1): R<sub>f</sub> 0.45. UV (MeOH): 258 (4.16), 232 (4.24), 205 (4.78). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 8.30 (s, H-C(8)); 8.04 (s, H-C(2)); 7.31 (m, 12 H, MeOTr); 6.82 (d, 2 H o to MeO); 6.16 (d, H-C(1')); 5.80 (s, NH<sub>2</sub>); 4.51 (t, H-C(3')); 4.41 (t, H-C(2')); 4.21 (q, H-C(4')); 3.78 (s, MeOTr); 3.56 (s, MeO-C(2')); 3.50 (dd, H-C(5')); 3.43 (dd, 1 H-C(5')); 2.95 (br., OH-C(3')). Anal. calc. for C<sub>31</sub>H<sub>31</sub>N<sub>5</sub>O<sub>5</sub> (553.6): C 67.26, H 5.64, N 12.65; found: C 67.13, H 6.02, N 11.49.

**22:** TLC (SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 20:1): R<sub>f</sub> 0.36. M.p. 99° (acetone) ([69]: 99–101°). UV (MeOH): 258 (4.24), 233 (4.19), 205 (4.79). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 8.29 (s, H-C(8)); 8.02 (s, H-C(2)); 7.28 (m, 12 H, MeOTr); 6.80 (d, 2 H o to MeO); 5.96 (d, H-C(1')); 5.62 (br., NH<sub>2</sub>); 4.87 (q, H-C(2')); 4.33 (q, H-C(4'), OH-C(2')); 4.06 (m, H-C(3')); 3.78 (s, MeOTr); 3.48 (s, MeO-C(3')); 3.46 (dd, 1 H-C(5')); 3.30 (dd, 1 H-C(5')). Anal. calc. for C<sub>31</sub>H<sub>31</sub>N<sub>5</sub>O<sub>5</sub> (553.6): C 67.26, H 5.64, N 12.65; found: C 67.09, H 5.89, N 12.04.

**N<sup>6</sup>-Methyl-N<sup>6</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (19).** Freshly prepared 0.73M CH<sub>2</sub>N<sub>2</sub> soln. (3 ml) was added dropwise to a soln. of N<sup>6</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**17**) [61] [63] (463 mg, 1.0 mmol) in DMF (30 ml) at 0°. After stirring for 30 h at 0°, the solvent was evaporated and the residue dried under high vacuum, co-evaporated with CH<sub>2</sub>Cl<sub>2</sub>, and again dried under high vacuum. The residue was purified by FC (silica gel, 15 g, 11 × 2 cm, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 100:3 (200 ml), 100:4 (100 ml), and 100:5 (260 ml)): 341 mg (72%) of **19**. Colorless foam. Physical data are in accordance to those found by Sigmund [64]. TLC (SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1): R<sub>f</sub> 0.55. UV (pH 3): 274 (4.30). <sup>1</sup>H-NMR ((D<sub>2</sub>O)DMSO): 10.63 (s, NH); 8.74 (s, H-C(8)); 8.71 (s, H-C(2)); 8.04 (d, 2 H o to NO<sub>2</sub>); 7.32 (d, 2 H m to NO<sub>2</sub>); 6.00 (d, H-C(1')); 5.53 (d, OH-C(2')); 5.27 (d, OH-C(3')); 5.11 (t, OH-C(5')); 4.61 (q, H-C(2')); 4.37 (m, CH<sub>2</sub>O); 4.15 (q, H-C(3')); 3.97 (m, H-C(4')); 3.55 (m, 2 H-C(5')); 3.60 (s, MeN); 2.98 (t, ArCH<sub>2</sub>). Anal. calc. for C<sub>20</sub>H<sub>22</sub>N<sub>6</sub>O<sub>8</sub> (474.4): C 50.63, H 4.67, N 17.71; found: C 49.96, H 4.85, N 17.35.

**2'- and 3'-O-Methylcytidine (24 and 25, resp.)** [42]. To a mixture of dry cytidine (**23**; 5.00 g, 20.6 mmol) and SnCl<sub>2</sub> · 2 H<sub>2</sub>O (135 mg, 0.6 mmol) in MeOH (1.0 l), a freshly prepared 0.75M CH<sub>2</sub>N<sub>2</sub> soln. (70 ml) was added dropwise at r.t. within 20 min (TLC: complete conversion). The solvent was evaporated and the residue dried under

high vacuum.  $^1\text{H-NMR}$ : **24/25** 82:18. The crude product was used for the synthesis of **26** without further purification.

*2'-O-Methyl-N<sup>4</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine (26)*. As described for **12**, with **24/25** 82:18 (5.4 g, 20.6 mmol), hexamethyldisilazane (45.0 ml, 34.8 g, 215 mmol),  $(\text{NH}_4)_2\text{SO}_4$ , dioxane (50 ml) (3 h; TLC ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  9:1)): complete conversion of **24/25** ( $R_f$  0.05) into the persilylated products ( $R_f$  0.51 and 0.54), then  $\text{CH}_2\text{Cl}_2$  (90 ml) and 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1*H*-imidazol-3-ium chloride (7.8 g, 25 mmol). The residue was stirred in MeOH (150 ml) and  $\text{Et}_3\text{N}$  (30 ml) overnight at r.t. The precipitated **26** (free of **27**) was filtered by suction, washed with MeOH, then with  $\text{Et}_2\text{O}$ , and dried under high vacuum at 40°: 6.30 g (68%) of **26**. Colorless powder. TLC ( $\text{SiO}_2$ ,  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  20:1);  $R_f$  0.26. M.p. 125° (MeOH). UV (MeOH): 281 (4.18), 243 (4.27), 212 (4.44).  $^1\text{H-NMR}$  ( $(\text{D}_6)$ DMSO): 10.79 (*s*, NH); 8.43 (*d*, H-C(6)); 8.15 (*d*, 2 H *o* to  $\text{NO}_2$ ); 7.59 (*d*, 2 H *m* to  $\text{NO}_2$ ); 6.97 (*d*, H-C(5)); 5.82 (*d*, H-C(1')); 5.20 (*t*, OH-C(5')); 5.10 (*t*, OH-C(3')); 4.34 (*t*,  $\text{CH}_2\text{O}$ ); 4.04 (*m*, H-C(3')); 3.86 (*d*, H-C(2')); 3.74 (*m*, 1 H-C(5')); 3.68 (*m*, H-C(4')); 3.58 (*m*, H-C(5')); 3.44 (*s*, MeO-C(2')); 3.07 (*t*,  $\text{ArCH}_2$ ). Anal. calc. for  $\text{C}_{19}\text{H}_{22}\text{N}_4\text{O}_9$  (450.4): C 50.67, H 4.92, N 12.44; found: C 50.21, H 5.16, N 12.24.

The filtrate was evaporated and twice co-evaporated with MeOH and the residue purified by FC (silica gel, 150 g, 22.5 × 4.5 cm,  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  100:1 (packed), 100:3 (400 ml), 100:4 (300 ml), 100:5 (300 ml), 100:6 (500 ml), and 100:7 (200 ml)): 1.94 g (21%) of **26/27**.  $^1\text{H-NMR}$ : **26/27** 24:76. Separation of the isomers was achieved after introducing the trityl group (see preparation of **28**, and **29**, method c)).

*3'-O-Methyl-N<sup>4</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine (27)*. As described for **12**, with 3'-*O*-methylcytidine (**25**) [42] (257 mg, 1.0 mmol), hexamethyldisilazane (2.3 ml, 1.8 g, 11 mmol),  $(\text{NH}_4)_2\text{SO}_4$ , dioxane (2.5 ml) (3.5 h; TLC ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  9:1)): complete conversion of **25** ( $R_f$  0.05) into the persilylated product ( $R_f$  0.54), then  $\text{CH}_2\text{Cl}_2$  (7.5 ml) and 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1*H*-imidazol-3-ium-chloride (375 mg, 1.20 mmol). The residue was stirred in MeOH (20 ml) and  $\text{Et}_3\text{N}$  (2.5 ml) overnight at r.t. The soln. was evaporated and purified by FC (silica gel, 15 g, 12 × 2.5 cm,  $\text{CH}_2\text{Cl}_2$  (400 ml),  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  100:2 (100 ml), 100:3 (100 ml), and 100:5 (300 ml)): 382 mg (85%) of **27**. Colorless amorphous solid. TLC ( $\text{SiO}_2$ ,  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  20:1);  $R_f$  0.24. M.p. 125–126° (MeOH). UV (MeOH): 281 (4.16), 242 (4.26), 212 (4.43).  $^1\text{H-NMR}$  ( $(\text{D}_6)$ DMSO): 10.76 (*s*, NH); 8.40 (*d*, H-C(6)); 8.15 (*d*, 2 H *o* to  $\text{NO}_2$ ); 7.59 (*d*, 2 H *m* to  $\text{NO}_2$ ); 6.96 (*d*, H-C(5)); 5.73 (*d*, H-C(1')); 5.51 (*d*, OH-C(2')); 5.25 (*br.*, OH-C(5')); 4.35 (*t*,  $\text{CH}_2\text{O}$ ); 4.18 (*t*, H-C(2')); 3.98 (*m*, H-C(4')); 3.68 (*m*, 1 H-C(5'), H-C(3')); 3.55 (*dd*, 1 H-C(5')); 3.30 (*s*, MeO-C(3')); 3.07 (*t*,  $\text{ArCH}_2$ ). Anal. calc. for  $\text{C}_{19}\text{H}_{22}\text{N}_4\text{O}_9$  (450.4): C 50.67, H 4.92, N 12.44; found: C 49.91, H 5.42, N 12.47.

*2'-O-Methyl-5'-O-(monomethoxytrityl)-N<sup>4</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine (28) and 3'-O-Methyl-5'-O-(monomethoxytrityl)-N<sup>4</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine (29)*. a) To a soln. of 5'-*O*-(monomethoxytrityl)-N<sup>4</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine (**34**) [61] [63] (3.54 g, 5.0 mmol) and  $\text{SnCl}_2 \cdot 2 \text{H}_2\text{O}$  (50 mg, 0.22 mmol) in  $\text{CH}_2\text{Cl}_2$  (250 ml), freshly prepared 0.45*M*  $\text{CH}_2\text{N}_2$  soln. (20 ml) was added dropwise at r.t. within 10 min (TLC: complete conversion). The solvent was evaporated and the residue dried under high vacuum.  $^1\text{H-NMR}$ : **28/29** 93:7. Separation of the isomers was achieved by FC (silica gel, 125 g, 18 × 4.5 cm, toluene/AcOEt 1:2 (0.85 l), 1:3 (1.2 l, **28**), 1:4 (1 l, **28**), 1:9 (0.5 l, **29**), AcOEt (0.25 l), and AcOEt/MeOH 20:1 (0.5 l)): 3.14 g (87%) of **28** and 180 mg (5%) **29**. Colorless foams.

b) As described for **14** (see a)), with **26** (9.0 g, 20 mmol), pyridine (50 ml), and  $\text{MeOTrCl}$  (8.0 g, 26 mmol) (quenching with MeOH). Workup with  $\text{CH}_2\text{Cl}_2$  (150 ml), phosphate buffer (pH 7, 150 ml),  $\text{CH}_2\text{Cl}_2$  (75 ml), and phosphate buffer (pH 7, 75 ml). FC (silica gel, 250 g, 25 × 6 cm, toluene/AcOEt 1:3 (500 ml), 1:4 (1000 ml), 1:6 (1000 ml), 1:8 (450 ml), 1:10 (450 ml), and AcOEt (600 ml)) gave 13.9 g (96%) of **28**. Colorless foam.

c) As described for **14** (see a)), with **26/27** (1.94 g, 4.31 mmol) pyridine (20 ml), and  $\text{MeOTrCl}$  (1.7 g, 5.6 mmol) (quenching with MeOH). Workup with  $\text{CH}_2\text{Cl}_2$  (80 ml), phosphate buffer (pH 7, 80 ml),  $\text{CH}_2\text{Cl}_2$  (40 ml), and phosphate buffer (pH 7, 40 ml). FC (silica gel, 100 g, 25 × 4 cm, toluene/AcOEt 1:3 (300 ml), 1:4 (500 ml, **28**), 1:6 (500 ml), 1:8 (450 ml, **29**), 1:10 (450 ml), and AcOEt (400 ml)) gave 595 mg (4% rel. to **23**) of **28** and 2.23 g (15% rel. to **23**) of **29**. Colorless foams.

d) As described for **14** (see a)), with **27** (1.35 g, 3.0 mmol), pyridine (10 ml), and  $\text{MeOTrCl}$  (1.1 g, 3.6 mmol). Workup with  $\text{CH}_2\text{Cl}_2$  (150 ml), phosphate buffer (pH 7, 50 ml),  $\text{CH}_2\text{Cl}_2$  (25 ml), and phosphate buffer (pH 7, 25 ml). FC (silica gel, 50 g, 14 × 3.5 cm, toluene/AcOEt 1:5 (200 ml), 1:7 (200 ml), 1:9 (200 ml), and AcOEt (200 ml)) gave 1.93 g (89%) of **29**. Colorless foam.

**28**: TLC ( $\text{SiO}_2$ ,  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  20:1);  $R_f$  0.53. UV (MeOH): 281 (4.21), 235 (4.43), 204 (4.76).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ): 8.52 (*d*, H-C(6)); 8.18 (*d*, 2 H *o* to  $\text{NO}_2$ ); 7.85 (*br.*, NH); 7.44–7.23 (*m*, 14 H,  $\text{MeOTr}$ , 2 H *m* to  $\text{NO}_2$ ); 6.86 (*d*, 2 H *o* to MeO); 6.82 (*d*, H-C(5)); 5.98 (*s*, H-C(1')); 4.43 (*t*, H-C(3'),  $\text{CH}_2\text{O}$ ); 4.02 (*d*, H-C(2')); 3.81 (*s*,  $\text{MeOTr}$ ); 3.78 (*m*, H-C(4')); 3.73 (*s*, MeO-C(2')); 3.62 (*dd*, 1 H-C(5')); 3.54 (*dd*, 1 H-C(5')); 3.10 (*t*,  $\text{ArCH}_2$ ); 2.64 (*br.*, OH-C(3')). Anal. calc. for  $\text{C}_{39}\text{H}_{38}\text{N}_4\text{O}_{10}$  (722.8): C 64.81, H 5.30, N 7.75; found: C 65.31, H 5.74, N 7.17.

**29:** TLC (SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 20:1): R<sub>f</sub> 0.43. UV (MeOH): 280 (4.19), 235 (4.44), 205 (4.74). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 8.31 (*d*, H-C(6)); 8.18 (*d*, 2 H *o* to NO<sub>2</sub>); 7.89 (br., NH); 7.41–7.22 (*m*, 14 H, MeOTr, 2 H *m* to NO<sub>2</sub>); 6.97 (*d*, H-C(5)); 6.86 (*d*, 2 H *o* to MeO); 5.92 (*d*, H-C(1')); 4.43 (*t*, H-C(2'), CH<sub>2</sub>O); 4.28 (*m*, H-C(4')); 3.98 (*d*, H-C(3')); 3.81 (*s*, MeOTr); 3.73 (*d*, OH-C(2')); 3.56 (*dd*, 1 H-C(5')); 3.43 (*s*, MeO-C(3')); 3.39 (*dd*, 1 H-C(5')); 3.11 (*t*, ArCH<sub>2</sub>). Anal. calc. for C<sub>39</sub>H<sub>38</sub>N<sub>4</sub>O<sub>10</sub> (722.8): C 64.81, H 5.30, N 7.75; found: C 64.59, H 5.60, N 7.47.

**5'-O-(Dimethoxytrityl)-2'-O-methyl-N<sup>4</sup>-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine (30).** As described for **14**, (see *a*), with **26** (1.80 g, 4.0 mmol), pyridine (5 ml), and (MeO)<sub>2</sub>TrCl (1.63 g, 4.8 mmol). Workup with CH<sub>2</sub>Cl<sub>2</sub> (50 ml), phosphate buffer (pH 7, 50 ml), CH<sub>2</sub>Cl<sub>2</sub> (25 ml), and phosphate buffer (pH 7, 25 ml). FC (silica gel, 50 g, 14 × 3.5 cm, toluene/AcOEt 1:4 (200 ml), 1:5 (150 ml), 1:7 (150 ml), and 1:9 (100 ml)) gave 2.56 g (85%) of **30**. Colorless foam. TLC (SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 20:1): R<sub>f</sub> 0.53. UV (MeOH): 275 (4.23), 235 (4.54), 205 (4.87). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 8.55 (*d*, H-C(6)); 8.34 (br., NH); 8.15 (*d*, 2 H *o* to NO<sub>2</sub>); 7.44–7.22 (*m*, 11 H, (MeO)<sub>2</sub>Tr 2 H *m* to NO<sub>2</sub>); 6.86 (*d*, 4 H *o* to MeO, H-C(5)); 5.97 (*s*, H-C(1')); 4.41 (*t*, H-C(3'), CH<sub>2</sub>O); 4.03 (*d*, H-C(2')); 3.80 (*s*, (MeO)<sub>2</sub>Tr, H-C(4')); 3.71 (*s*, MeO-C(2')); 3.62 (*dd*, 1 H-C(5')); 3.54 (*dd*, 1 H-C(5')); 3.09 (*t*, ArCH<sub>2</sub>); 2.83 (br., OH-C(3')). Anal. calc. for C<sub>40</sub>H<sub>40</sub>N<sub>4</sub>O<sub>11</sub> (752.8): C 63.82, H 5.36, N 7.44; found: C 63.81, H 5.42, N 7.20.

**2'-O-Methyluridine (36).** Compound **39** (2.65 g, 5.0 mmol) was stirred in 80% aq. AcOH (15 ml) overnight at r.t. The mixture was evaporated and co-evaporated with MeOH until all acid was removed. The crude product was crystallized from MeOH (3 ml)/AcOEt (20 ml) to give 830 mg (64%) of **36**. Recrystallization from MeOH/AcOEt gave another 270 mg (21%) of **36**. Combined yield: 1.10 g (85%) of **36** [42]. TLC (SiO<sub>2</sub>, CHCl<sub>3</sub>/MeOH 5:1): R<sub>f</sub> 0.42. M.p. 163° (MeOH/AcOEt). UV (MeOH): 261 (3.99). <sup>1</sup>H-NMR (D<sub>2</sub>O): 7.88 (*d*, H-C(6)); 5.96 (*d*, H-C(1')); 5.87 (*d*, H-C(5)); 4.31 (*t*, H-C(3')); 4.05 (*m*, H-C(2'), H-C(4')); 3.90 (*dd*, 1 H-C(5')); 3.77 (*dd*, 1 H-C(5')); 3.49 (*s*, MeO-C(2')). Anal. calc. for C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>O<sub>6</sub> (258.2): C 46.51, H 5.46, N 10.85; found: C 46.19, H 5.41, N 10.80.

**3'-O-Methyluridine (37).** Compound **40** (500 mg, 0.94 mmol) was stirred in 2% TsOH in CH<sub>2</sub>Cl<sub>2</sub>/MeOH 4:1 (15 ml) at r.t. for 15 min. The mixture was neutralized with some drops of NaOMe soln. and evaporated. The residue was taken up in CH<sub>2</sub>Cl<sub>2</sub> and purified by FC (silica gel, 12 g, 13.5 × 2 cm, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 100:3 (250 ml), 100:4 (100 ml), and 100:5 (200 ml)): 202 mg (83%) of **37** [42]. Colorless glassy oil. TLC (SiO<sub>2</sub>, CHCl<sub>3</sub>/MeOH 5:1): R<sub>f</sub> 0.42. UV (MeOH): 261 (3.99). <sup>1</sup>H-NMR (D<sub>2</sub>O): 7.85 (*d*, H-C(6)); 5.88 (*d*, H-C(1')); 5.86 (*d*, H-C(5)); 4.48 (*t*, H-C(2')); 4.17 (*m*, H-C(4')); 3.90 (*m*, 1 H-C(5'), H-C(3')); 3.77 (*dd*, 1 H-C(5')); 3.45 (*s*, MeO-C(3')). Anal. calc. for C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>O<sub>6</sub> (258.2): C 46.51, H 5.46, N 10.85; found: C 46.25, H 5.47, N 10.82.

**2'-O-Methyl-5'-O-(monomethoxytrityl)uridine (39) and 3'-O-Methyl-5'-O-(monomethoxytrityl)uridine (40).** To a soln. of 5'-O-(monomethoxytrityl)uridine (**38**) [66] (5.17 g, 10.0 mmol) and SnCl<sub>2</sub> · 2 H<sub>2</sub>O (56 mg, 0.25 mmol) in dioxane (250 ml), freshly prepared 1M CH<sub>2</sub>N<sub>2</sub> soln. (20 ml) was added dropwise at r.t. within 20 min (TLC: complete conversion). The solvent was evaporated and the residue dried under high vacuum. <sup>1</sup>H-NMR: **39/40** 78:22. Separation of the isomers was achieved by FC (silica gel, 200 g, 19 × 5.5 cm, toluene/AcOEt 2:1 (1.1 l), 3:2 (2.5 l), **39**), 1:1 (1 l, **39**), 2:3 (0.5 l, **40**), 1:4 (0.5 l), and 1:6 (0.5 l)): 3.91 g (74%) of **39** [56] and 1.11 g (21%) of **40**. Colorless foams.

**39:** TLC (SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 20:1): R<sub>f</sub> 0.59. M.p. 103–106° (Et<sub>2</sub>O). UV (MeOH): 262 (3.98), 231 (4.19), 204 (4.72). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 9.38 (br., NH); 8.05 (*d*, H-C(6)); 7.41–7.21 (*m*, 12 H, MeOTr); 6.86 (*d*, 2 H *o* to MeO); 5.97 (*s*, H-C(1')); 5.26 (*d*, H-C(5)); 4.49 (*q*, H-C(3')); 4.00 (*d*, H-C(2')); 3.80 (*s*, MeOTr, H-C(4')); 3.65 (*s*, MeO-C(2')); 3.56 (*s*, 2 H-C(5')); 2.65 (*d*, OH-C(3')). Anal. calc. for C<sub>30</sub>H<sub>30</sub>N<sub>2</sub>O<sub>7</sub> (530.6): C 67.91, H 5.70, N 5.28; found: C 67.69, H 5.83, N 5.13.

**40:** TLC (SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 20:1): R<sub>f</sub> 0.49. M.p. 112–114° (EtOH). UV (MeOH): 261 (4.00), 231 (4.21), 204 (4.73). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 9.32 (br., NH); 7.83 (*d*, H-C(6)); 7.40–7.21 (*m*, 12 H, MeOTr); 6.86 (*d*, 2 H *o* to MeO); 5.93 (*d*, H-C(1')); 5.41 (*d*, H-C(5)); 4.36 (*q*, H-C(2')); 4.20 (*q*, H-C(4')); 3.98 (*t*, H-C(3')); 3.80 (*s*, MeOTr); 3.54 (*m*, 1 H-C(5'), OH-C(2')); 3.45 (*s*, MeO-C(3')); 3.42 (*dd*, 1 H-C(5')). Anal. calc. for C<sub>30</sub>H<sub>30</sub>N<sub>2</sub>O<sub>7</sub> (530.6): C 67.91, H 5.70, N 5.28; found: C 67.62, H 5.84, N 5.02.

**5'-O-(Dimethoxytrityl)-2'-O-methyluridine (42) and 5'-O-(Dimethoxytrityl)-3'-O-methyluridine (43).** To a soln. of 5'-O-(dimethoxytrityl)uridine (**41**) (2.73 g, 5.0 mmol) and SnCl<sub>2</sub> · 2 H<sub>2</sub>O (30 mg, 0.13 mmol) in dioxane (125 ml), freshly prepared 0.8M CH<sub>2</sub>N<sub>2</sub> soln. (10 ml) was added dropwise at r.t. within 20 min (TLC: complete conversion). The solvent was evaporated and the residue dried under high vacuum. <sup>1</sup>H-NMR: **42/43** 75:25. Separation of the isomers was achieved by FC (silica gel, 200 g, 19 × 5.5 cm, toluene/acetone 6:1 (1.2 l), 5:2 (1.2 l), **42**), 4:1 (0.7 l, **43**) and 3:1 (0.6 l)): 1.98 g (71%) of **42** and 601 mg (22%) of **43**. Colorless foams.

**42:** TLC (SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 20:1): R<sub>f</sub> 0.59. UV (MeOH): 264 (4.08), 234 (4.39), 205 (4.77). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 9.70 (br., NH); 8.05 (*d*, H-C(6)); 7.41–7.21 (*m*, 9 H, (MeO)<sub>2</sub>Tr); 6.84 (*d*, 4 H *o* to MeO); 5.98 (*d*, H-C(1')); 5.28 (*d*, H-C(5)); 4.48 (*q*, H-C(3')); 4.00 (*d*, H-C(2')); 3.79 (*s*, (MeO)<sub>2</sub>Tr, H-C(4')); 3.64 (*s*,

MeO–C(2')); 3.55 (*m*, 2 H–C(5')); 2.72 (*d*, OH–C(3')). Anal. calc. for C<sub>31</sub>H<sub>32</sub>N<sub>2</sub>O<sub>8</sub> (560.6): C 66.42, H 5.75, N 5.00; found: C 65.84, H 5.83, N 4.84.

**43:** TLC (SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 20:1): R<sub>f</sub> 0.49. UV (MeOH): 264 (4.07), 234 (4.38), 205 (4.76). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 9.60 (br., NH); 7.85 (*d*, H–C(6)); 7.40–7.21 (*m*, 9 H, (MeO)<sub>2</sub>Tr); 6.85 (*d*, 4 H *o* to MeO); 5.94 (*d*, H–C(1')); 5.42 (*d*, H–C(5)); 4.37 (*m*, H–C(2')); 4.21 (*m*, H–C(4')); 3.98 (*t*, H–C(3')); 3.79 (*s*, (MeO)<sub>2</sub>Tr, OH–C(2')); 3.54 (*dd*, 1 H–C(5')); 3.45 (*s*, MeO–C(3')); 3.39 (*dd*, 1 H–C(5')). Anal. calc. for C<sub>31</sub>H<sub>32</sub>N<sub>2</sub>O<sub>8</sub> (560.6): C 66.42, H 5.75, N 5.00; found: C 65.93, H 5.84, N 4.84.

**N<sup>3</sup>-Anisoyluridine (44)** [65]. A mixture of dry uridine (**35**; 1.22 g, 5.00 mmol), hexamethyldisilazane (11.5 ml, 55 mmol), and a catal. amount of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was heated under reflux in anh. dioxane (20 ml) for 3 h. After cooling, the mixture was evaporated and twice co-evaporated with anh. toluene. The residue was dissolved in anh. pyridine (15 ml), and (*i*-Pr)<sub>2</sub>EtN (1.28 ml, 7.5 mmol) and anisoyl chloride (1.36 ml, 1.7 g, 10 mmol) were added. After stirring for 3 h at r.t., the mixture was quenched with MeOH (2 ml), concentrated, diluted with CH<sub>2</sub>Cl<sub>2</sub> (50 ml), and washed with phosphate buffer (pH 7, 50 ml). The aq. phase was extracted twice with CH<sub>2</sub>Cl<sub>2</sub> (25 ml) and the combined org. phase washed with phosphate buffer (pH 7, 25 ml). The org. phase was dried (Na<sub>2</sub>SO<sub>4</sub>), evaporated, and co-evaporated 3× with toluene (5 ml). The residue was stirred in MeOH (30 ml) and Et<sub>3</sub>N (6 ml) overnight at r.t. The soln. was evaporated and co-evaporated twice with MeOH. The residue was taken up in MeOH (20 ml), silica gel (5 g), added, and the solvent evaporated. The residue was added on top of a silica-gel column (packed with toluene/AcOEt 4:1) and purified by FC (silica gel, 50 g, 13 × 3.5 cm, toluene/AcOEt 1:6 (150 ml), 1:8 (180 ml), 1:10 (150 ml), 1:15 (160 ml, **44**), 1:20 (210 ml), AcOEt (400 ml), and AcOEt/MeOH (210 ml)): 1.23 g (65%) of **44**. Colorless foam. TLC (SiO<sub>2</sub>, AcOEt/MeOH 20:1): R<sub>f</sub> 0.32. UV (MeOH): 282 (4.35), 218 (4.16), 203 (4.23). <sup>1</sup>H-NMR ((D<sub>6</sub>)DMSO): 8.14 (*d*, H–C(6)); 7.92 (*d*, 2 H *o* to CO); 7.09 (*d*, 2 H *o* to MeO of An); 5.91 (*d*, H–C(5)); 5.75 (*d*, H–C(1')); 5.49 (*d*, OH–C(2')); 5.18 (*t*, OH–C(5')); 5.12 (*d*, OH–C(3')); 4.10 (*m*, H–C(2')); 4.01 (*m*, H–C(3')); 3.86 (*s*, MeO of An, H–C(4')); 3.63 (*m*, 2 H–C(5')). Anal. calc. for C<sub>17</sub>H<sub>18</sub>N<sub>2</sub>O<sub>8</sub> (378.3): C 53.97, H 4.80, N 7.40; found: C 53.31, H 4.94, N 7.01.

**N<sup>3</sup>-Anisoyl-5'-O-(monomethoxytrityl)uridine (45)**. *a*) As described for **44**, with 5'-O-(monomethoxytrityl)uridine (**38**) [66] (1.29 g, 2.50 mmol), hexamethyldisilazane (4.2 ml, 20 mmol) (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, dioxane (10 ml), then pyridine (15 ml), (*i*-Pr)<sub>2</sub>EtN (0.64 ml, 3.75 mmol) and anisoyl chloride (0.68 ml, 0.85 g, 5 mmol). After co-evaporation with MeOH (2×) purification by FC (silica gel, 40 g, 12 × 3.5 cm, toluene/AcOEt 4:1 (150 ml), 3:1 (280 ml), and 2:1 (350 ml)) gave 1.15 g (71%) of **45** as yellow foam. Subsequent chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH) yielded a colorless foam.

*b*) As described for **14** (see *a*)), with **44** (1.99 g, 5.3 mmol), pyridine (15 ml), and MeOTrCl (1.98 g, 6.3 mmol) (48 h; quenching with MeOH). Workup with CH<sub>2</sub>Cl<sub>2</sub> (50 ml), phosphate buffer (pH 7, 50 ml), CH<sub>2</sub>Cl<sub>2</sub> (25 ml), and phosphate buffer (pH 7, 25 ml). FC (silica gel, 90 g, 21 × 3.5 cm, toluene/AcOEt 5:1 (250), 4:1 (250), 3:1 (400), 5:2 (350), 2:1 (300), and 1:1 (200)) gave 2.51 g (73%) of **45**. Colorless foam. TLC (SiO<sub>2</sub>; toluene/AcOEt 1:1): R<sub>f</sub> 0.28. UV (MeOH): 281 (4.43), 222 (sh, 4.48), 205 (4.85). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 7.90 (*d*, H–C(6)); 7.88 (*d*, 2 H *o* to CO); 7.41–7.23 (*m*, 12 H, MeOTr); 6.93 (*d*, 2 H *o* to MeO of An); 6.86 (*d*, 2 H *o* to MeO); 5.79 (*d*, H–C(1')); 5.75 (*d*, H–C(5)); 4.39 (*m*, H–C(2')); 4.34 (*m*, H–C(3')); 4.21 (*m*, H–C(4')); 4.04 (br., OH–C(3')); 3.85 (*s*, MeO of An); 3.80 (*s*, MeOTr); 3.49 (*m*, 1 H–C(5')); 3.43 (*m*, 1 H–C(5')); 3.00 (br., OH–C(2')). Anal. calc. for C<sub>37</sub>H<sub>38</sub>N<sub>2</sub>O<sub>9</sub> (650.7): C 68.30, H 5.89, N 4.30; found: C 68.09, H 5.51, N 4.18.

**N<sup>3</sup>-Anisoyl-2'-O-methyl-5'-O-(monomethoxytrityl)uridine (46)** and **N<sup>3</sup>-Anisoyl-3'-O-methyl-5'-O-(monomethoxytrityl)uridine (47)**. To a soln. of **45** (3.26 g, 5.0 mmol) and SnCl<sub>2</sub> · 2 H<sub>2</sub>O (30 mg, 0.13 mmol) in dioxane (125 ml), freshly prepared 1.0M CH<sub>2</sub>N<sub>2</sub> soln. (10 ml) was added dropwise at r.t. within 10 min (TLC: complete conversion). The solvent was evaporated and the residue dried under high vacuum. <sup>1</sup>H-NMR: **46/47** 70:30. Separation of the isomers was achieved by FC (silica gel, 100 g, 14 × 5 cm, toluene/acetone 12:1 (0.3 l), 10:1 (1 l, **46**), 8:1 (0.3 l, **47**), and 6:1 (0.3 l)): 2.15 g (65%) of **46** and 945 mg (28%) of **47**. Colorless foams.

**46:** TLC (SiO<sub>2</sub>, toluene/AcOEt 5:4): R<sub>f</sub> 0.63. UV (MeOH): 281 (4.37), 223 (sh, 4.47), 205 (4.79). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 8.18 (*d*, H–C(6)); 7.90 (*d*, 2 H *o* to CO); 7.43–7.18 (*m*, 12 H, MeOTr); 6.95 (*d*, 2 H *o* to MeO of An); 6.87 (*d*, 2 H *o* to MeO); 5.93 (*s*, H–C(1')); 5.33 (*d*, H–C(5)); 4.53 (*m*, H–C(3')); 3.99 (*m*, H–C(2')); 3.86 (*s*, MeO of An); 3.80 (*s*, MeOTr); 3.83 (*m*, H–C(4')); 3.60 (*m*, 2 H–C(5'), MeO–C(2')); 2.65 (br., OH–C(3')). Anal. calc. for C<sub>38</sub>H<sub>36</sub>N<sub>2</sub>O<sub>4</sub> (664.7): C 68.66, H 5.46, N 4.21; found: C 68.35, H 5.55, N 4.16.

**47:** TLC (SiO<sub>2</sub>, toluene/AcOEt 5:4): R<sub>f</sub> 0.46. UV (MeOH): 281 (4.35), 222 (sh, 4.47), 205 (4.78). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 7.90 (*d*, H–C(6), 2 H *o* to CO); 7.42–7.26 (*m*, 12 H, MeOTr); 6.88 (*m*, 4 H *o* to MeO); 5.87 (*s*, H–C(1')); 5.50 (*d*, H–C(5)); 4.41 (*m*, H–C(2')); 4.16 (*m*, H–C(4')); 4.04 (*t*, H–C(3')); 3.83 (*s*, MeO of An); 3.81 (*s*, MeOTr); 3.58 (*dd*, 1 H–C(5')); 3.42 (*s*, MeO–C(3')); 3.38 (*dd*, 1 H–C(5')); 2.92 (br., OH–C(2')). Anal. calc. for C<sub>38</sub>H<sub>36</sub>N<sub>2</sub>O<sub>4</sub> (664.7): C 68.66, H 5.46, N 4.21; found: C 68.51, H 5.52, N 4.26.

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