78. Nucleotides

Part LII[1])

Synthesis and Biological Activity of New Base-Modified (2′–5′)Oligoadenylate Trimers

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Some new (2′–5′)oligoadenylate trimers, *i.e.*, **22**–**28**, containing the antiviral nucleoside ribavirin (= 1-(β-D-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide; **7**) and the synthetic cytokine 6-(benzylamino)purine riboside (= N^6-benzyladenosine; **1**) in different positions of the trimer, have been synthesized by the phosphotriester method. The selectively blocked nucleosides **2**–**6** and **8**–**11** and the 2′-phosphodiesters **13** and **14**, used for the oligonucleotide syntheses, were synthesized from the corresponding unprotected ribonucleosides **1** and **7**, and isolated by silica-gel column chromatography. The fully deblocked trimers **22**–**28** were purified by ion-exchange chromatography on *DEAE-Servacell 23-SS*. The newly synthesized compounds were characterized by physical means. The ability of synthesized trimers to inhibit HIV-1 replication and to improve RNase L activation were investigated. Some of the synthesized trimers showed also biological inhibition of HIV-1 reverse transcriptase and HIV-1-induced syncytia formation. It was shown that Ado^{Bn}-containing trimers inhibited HIV-1-induced syncytia formation > 1500-fold, independently of the position of the Ado^{Bn} residue in the oligomer chain.

1. Introduction. – The discovery of the (2′–5′)oligoadenylates is connected with the study of the mechanisms of interferon action as the cellular response to virus infection [2]. The 5′-triphosphate of (2′–5′)oligoadenylate trimer plays the most important role in the antiviral mechanism induced by interferon [3]. Furthermore, naturally occurring (2′–5′)oligoadenylates (both, 5′-phosphorylated and unphosphorylated) have shown different kinds of biological activity [4][5]. Many analogues of the natural (2′–5′)oligoadenylates have been synthesized to achieve new approaches to antiviral and antitumoral therapy [6–13]. Biological activities of 5′-phosphorylated (2′–5′)-oligoadenylates are connected with the functioning of the (2′–5′)A system which is finally leading to the inhibition of protein synthesis [3]. The mechanism of action of unphosphorylated (2′–5′)oligoadenylates in many cases is still unknown. Recently, some of the sugar-modified trimers of (2′–5′)oligoadenylates were found to be inhibitors of HIV-1

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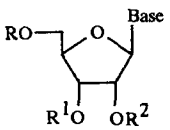
reverse transcriptase (RT) [14–18]. As far as each individual nucleoside residue of (2′–5′)oligoadenylates may assume a different role in inhibition of RT or RNase L activation, we synthesized new types of (2′–5′)oligonucleotide trimers containing the known antiviral nucleoside 1-(β-D-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide; **7** (ribavirin) and the cytokine 6-(benzylamino)purine riboside (= N^6-benzyladenosine; **1**) in different positions of the trimer instead of the adenosine residue. The potential of the new trimers to inhibit HIV-1 replication and to improve RNase L activation were investigated.

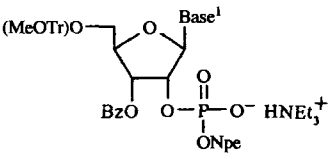
2. Syntheses. – The syntheses of new (2′–5′)oligonucleotide trimers were achieved by the phosphotriester method using the approach published by us earlier [19]. Synthetic N^6-benzyladenosine (**1**) and 1-(β-D-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide (**7**), obtained by the reaction of microbiological transglycosylation as described before [20], were converted into the corresponding selectively blocked nucleosides **2**–**6** and **8**–**11** and corresponding nucleotides **13** and **14**, respectively. Thus, treatment of **1** with monomethoxytrityl chloride (MeOTrCl) in pyridine gave the 5′-*O*-monomethoxytrityl derivative **2** (85%). Benzoylation of **2** with benzoyl cyanide (BzCN) in MeCN [19] led to a mixture of the 2′,3′-di-*O*-benzoyl (**3**), 2′-*O*-benzoyl (**4**), and 3′-*O*-benzoyl (**5**) derivatives which were isolated by column chromatography (CC) in 38, 13, and 46% yield, respectively. Treatment of **3** with a 2% solution of TsOH in CH_2Cl_2/MeOH 7:3 afforded 2′,3′-di-*O*-benzoyl-N^6-benzyladenosine (**6**) in 96% yield. Similarly, **7** was converted into the 5′-*O*-monomethoxytrityl derivatives **8**–**10** in 92, 16, and 57% yield, respectively. Detritylation of **9** led to the 5′-OH derivative **11** in 78% yield. Furthermore, the reaction of the 3′-*O*-benzoylated compounds **5** and **10** with 2-chlorophenyl bis(1*H*-1,2,4-triazol-1-yl)-phosphinate followed by subsequent treatment with 2-(4-nitrophenyl)-ethanol (NpeOH) and then a solution of 4-nitrobenzaldoxim in dioxane/H_2O/Et_3N 1:1:1, gave the corresponding nucleoside 2′-phosphodiesters **13** and **14** which were isolated by CC (silica gel) in 82 and 48% yield, respectively.

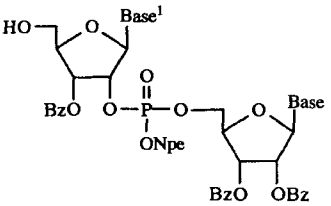
The compounds **6**, **11**, **13**, and **14** and the corresponding adenosine derivatives **12** [21] and **15** [22] were then used in the syntheses of the new (2′–5′)oligonucleotide trimers **22**–**29**. Condensation of 2′,3′-di-*O*-benzoyl-N^6-benzyladenosine (**6**) with the 2′-phosphodiester **15** in pyridine in the presence of a mixture of 1*H*-triazole/2,4,6-triisopropylbenzenesulfonyl chloride (TpsCl) 3:1, followed by detritylation, led to the 5′-OH dimer **16** which was isolated by CC in 85% yield. Similar reaction sequences converted compounds **6** and **13**, **12** and **13**, **11** and **15**, and **12** and **14**, into the corresponding 5′-OH dimers **17**–**20**, isolated in 81, 87, 74, and 76% yield, respectively. The synthesis of 5′-OH dimer **21** has been described by us earlier [22].

The transformations of the dimers **16**–**20** to the trimer level afforded the same techniques consisting of a condensation step and followed by successive treatment with 2% solution of TsOH, 0.5M DBU (1,8-diazabicyclo[5.4.0]undec-7-ene)/pyridine, and NH_3/MeOH, respectively, to remove the three different protecting groups. Final purification by CC (*DEAE*-cellulose)ion exchange gave the trimers **22**–**28** in good to moderate yields.

3. Biological Application. – Replacement of the adenine moiety in the (2′–5′)-oligoadenylate trimer core with 1*H*-1,2,4-triazole-3-carboxamide (TCA) or with



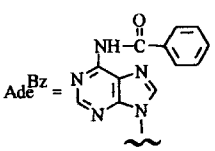


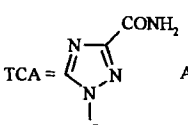


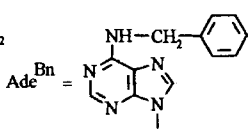
	Base	R	R^1	R^2
1	Ade^{Bn}	H	H	H
2	Ade^{Bn}	MeOTr	H	H
3	Ade^{Bn}	MeOTr	Bz	Bz
4	Ade^{Bn}	MeOTr	H	Bz
5	Ade^{Bn}	MeOTr	Bz	H
6	Ade^{Bn}	H	Bz	Bz
7	TCA	H	H	H
8	TCA	MeOTr	H	H
9	TCA	MeOTr	Bz	Bz
10	TCA	MeOTr	Bz	H
11	TCA	H	Bz	Bz
12	Ade^{Bz}	H	Bz	Bz

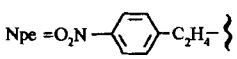
	$Base^1$
13	Ade^{Bn}
14	TCA
15	Ade^{Bz}

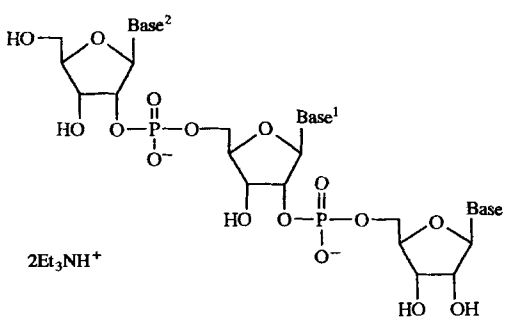
	Base	$Base^1$
16	Ade^{Bn}	Ade^{Bz}
17	Ade^{Bn}	Ade^{Bn}
18	Ade^{Bz}	Ade^{Bn}
19	TCA	Ade^{Bz}
20	Ade^{Bz}	TCA
21	Ade^{Bz}	Ade^{Bz}











	Base	$Base^1$	$Base^2$
22	Ade^{Bn}	Ade	Ade
23	Ade	Ade^{Bn}	Ade
24	Ade	Ade	Ade^{Bn}
25	Ade^{Bn}	Ade^{Bn}	Ade^{Bn}
26	TCA	Ade	Ade
27	Ade	TCA	Ade
28	Ade	Ade	TCA
29	Ade	Ade	Ade

N^6-(benzylamino)purine (Ade^{Bn}) moieties produced a new group of inhibitors of HIV-1 replication. The three studies performed to determine the antiviral activity of these (2′–5′)oligonucleotides were *i*) inhibition of HIV-1-induced syncytia formation, *ii*) inhibition of HIV-1 reverse-transcriptase (RT) activity, and *iii*) activation of recombinant human GST-RNase L. The TCA-containing trimers **26**–**28** inhibited HIV-1 replication to the same extent as naturally occurring trimer **29**, as determined by the inhibition of HIV-1-induced syncytia formation (*Table*). In contrast to the trimers **26**–**28**, the Ade^{Bn}-containing trimers **22**–**25** inhibited HIV-1-induced syncytia formation > 1500-fold. On the other hand, the TCA-containing trimers **26**–**28** inhibited HIV-1 RT activity by 99.7, 99.7, and 99.5%, respectively; however, the inhibition of HIV-1 RT activity by the Ade^{Bn}-containing trimers **22**–**25** was dependent on the position of the Ade^{Bn} group in the oligonucleotide chain. The trimer **22**, being N^6-benzyl-substituted at the 5′-terminus of the oligomer, inhibited HIV-1 RT activity by 33% compared to the trimers **23**–**25**, which inhibited HIV-1 RT activity by 7.6, 16.7, and 10.6%. The TCA- and Ade^{Bn}-containing (2′–5′)trimers inhibited recombinant human GST-RNase L activity as a function of the change in structure of the base moiety. The (2′–5′)trimer **26** with the TCA moiety at the 5′-terminus activated GST-RNase L by 87.7%, compared to 50% hydrolysis of poly(U)-3′-[^{32}P]pCp with naturally occurring trimer **29**. The (2′–5′)trimers **22**–**25** with the Ade^{Bn} moiety instead of adenine activated GST-RNase L by 37.4, 34.8, 0, and 13.6%, respectively.

Table. *Inhibition of HIV-1-Replication and Biological Activities of (2′–5′)Oligonucleotide Trimers* **22**–**29**[a])

	Base	Base[1]	Base[2]	Syn.[b])	RT[c])	RNase L[d])
22	Ade^{Bn}	Ade	Ade	>1500	33	37.4
23	Ade	Ade^{Bn}	Ade	>1500	7.6	34.8
24	Ade	Ade	Ade^{Bn}	>1500	16.7	0
25	Ade^{Bn}	Ade^{Bn}	Ade^{Bn}	>1500	10.6	13.6
26	TCA	Ade	Ade	1.6	99.7	87.7
27	Ade	TCA	Ade	7.0	99.7	9.4
28	Ade	Ade	TCA	1.2	99.5	0
29	Ade	Ade	Ade	3.0	33	50

[a]) Compounds were tested at 300 μM.

[b]) Inhibition of HIV-1 replication was determined by HIV-1-induced syncytia formation (fold reduction) for each compound. The number of syncytia/10^4 cells was 121 ± 16 for the control Sup T1 cells. The mean of triplicate determinations is shown; variance did not exceed 5–10%.

[c]) Percent inhibition of reverse-transcriptase (HIV-1 RT) activity. Control values for HIV-1 RT activity ranged from 15000 to 16000 cpm. The mean of duplicate determinations is shown; variance did not exceed 5–10%.

[d]) The activation of recombinant human RNase L was measured as the percent hydrolysis of poly(U)-3′-[^{32}P]pCp in the presence of the trimers **22**–**29**. The mean of duplicate determinations is shown; variance did not exceed 5–10%.

These data support the hypothesis that the adenine moiety at the 2′,3′-terminus of the (2′–5′)oligoadenylate trimer core **29** is essential for the activation of recombinant human RNase L.

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Experimental Part

General. TLC: Precoated silica gel thin-layer sheets *60 F 254* from *Merck*. Prep. column chromatography (CC): silica gel (*Merck 60*, 63–200 μm). Ion-exchange chromatography: *DEAE-Servacel 23-SS* (*Serva*). M.p.: *Gallenkamp* melting-point apparatus; no correction. UV/VIS: *Specord UV-VIS* (*Carl Zeiss*, Germany); λ_{max} in nm (log ε). ^{1}H-NMR: *Bruker WM-360*; δ in ppm rel. to $SiMe_4$.

Bioassay. Assays measuring HIV-1-induced syncytia formation, HIV-1 reverse transcriptase activity, and activation of RNase L were accomplished as described [23].

N^6-Benzyl-5'-O-(monomethoxytrityl)adenosine (**2**). To a soln. of *N*^{6}-benzyladenosine (**1**, 1.67 g, 4.67 mmol) in pyridine (17 ml), 4-methoxytrityl chloride (2 g, 6.54 mmol) was added at r.t. The mixture was stirred at r.t. for 20 h and then added dropwise to a mixture of H_2O and ice (800 g). The precipitate was filtered off, dissolved in $CHCl_3$ (150 ml), and washed with H_2O (2 × 40 ml). The org. layer was dried (Na_2SO_4) and evaporated. The residue was purified by CC (silica gel, 15 × 3.5 cm, $CHCl_3$, then $CHCl_3$/MeOH 20:1) and finally crystallized from EtOH: 2.5 g (85%) of **2**. M.p. 165–167°. UV (MeOH): 234 (4.21), 271 (4.30). ^{1}H-NMR ((D$_6$)DMSO): 8.40 (*s*, NH); 8.27, 8.13 (2*s*, H–C(2), H–C(8)); 7.37–6.82 (*m*, 19 arom. H); 5.93 (*dd*, H–C(1')); 5.55 (*d*, OH–C(2')); 5.22 (*d*, OH–C(3')); 4.70 (*s*, PhCH_2); 4.36 (*dd*, H–C(2')); 4.30 (*m*, H–C(3')); 4.05 (*m*, H–C(4')); 3.71 (*s*, MeO); 3.21 (*d*, 2 H–C(5')). Anal. calc. for $C_{37}H_{35}N_5O_5$ (629.7): C 70.57, H 5.60, N 11.12; found: C 70.43, H 5.56, N 11.21.

2',3'-Di-O-benzoyl-N^6-benzyl-5'-O-(monomethoxytrityl)adenosine (**3**), *2'-O-Benzoyl-N^6-benzyl-5'-O-(monomethoxytrityl)adenosine* (**4**), *and 3'-O-Benzoyl-N^6-benzyl-5'-O-(monomethoxytrityl)adenosine* (**5**). A soln. of benzoyl cyanide (0.27 g, 2.06 mmol) in MeCN (20 ml) was added at r.t. within 30 min to a soln. of **2**, Et_3N (2.9 ml), and 4-(dimethylamino)pyridine (DMAP; 50 mg) in MeCN (30 ml). The mixture was stirred at r.t. for 18 h and evaporated. Purification by CC (silica gel, 30 × 3.5 cm, hexane/AcOEt 4:1 → 1:4) gave, after drying under high vacuum, 0.5 g (38%) of **3**, 0.15 g (13%) of **4**, and 0.54 g (46%) of **5** as colorless foams.

3: UV (MeOH): 232 (4.60), 272 (4.36). ^{1}H-NMR ((D$_6$)DMSO): 8.53 (*s*, NH); 8.38, 8.17 (2*s*, H–C(2), H–C(8)); 7.94–6.79 (*m*, 29 arom. H); 6.52 (*d*, H–C(1')); 6.47 (*dd*, H–C(2')); 6.20 (*dd*, H–C(3')); 4.72 (*s*, PhCH_2); 4.60 (*m*, H–C(4')); 3.68 (*s*, MeO); 3.43 (*m*, 2 H–C(5')). Anal. calc. for $C_{51}H_{43}N_5O_7$ (837.9): C 73.10, H 5.17, N 8.35; found: C 73.32, H 5.20, N. 8.24.

4: UV (MeOH): 233 (4.45), 272 (4.32). ^{1}H-NMR ((D$_6$)DMSO): 8.50 (*s*, NH); 8.36, 8.20 (2*s*, H–C(2), H–C(8)); 8.07–6.83 (*m*, 24 arom. H); 6.37 (*d*, H–C(1')); 6.09 (*dd*, H–C(2')); 5.75 (*d*, OH–C(3')); 4.90 (*dd*, H–C(3')); 4.72 (*s*, PhCH_2); 4.22 (*m*, H–C(4')); 3.71 (*s*, MeO); 3.30 (*m*, 2 H–C(5')). Anal. calc. for $C_{44}H_{39}N_5O_6$ (733.8): C 72.01, H 5.35, N 9.54; found C 72.18, H 5.30, N 9.37.

5: UV (MeOH): 233 (4.48), 272 (4.34). ^{1}H-NMR ((D$_6$)DMSO): 8.47 (*s*, NH); 8.35, 8.14 (2*s*, H–C(2), H–C(8')); 8.07-6.80 (*m*, 24 arom. H); 6.04 (*d*, H–C(1')); 5.98 (*d*, OH–C(2')); 5.64 (*dd*, H–C(3')); 5.23 (*dd*, H–C(2')); 4.72 (*s*, PhCH_2); 4.39 (*m*, H–C(4')); 3.67 (*s*, MeO); 3.36 (*m*, 2 H–C(5')). Anal. calc. for $C_{44}H_{39}N_5O_6$ (733.8): C 72.01, H 5.35, N 9.54; found: C 72.20, H 5.40, N 9.40.

2',3'-Di-O-benzoyl-N^6-benzyladenosine (**6**). A soln. of **3** (0.1 g, 0.12 mmol) was stirred with 2% TsOH in CH_2Cl_2/MeOH 7:3 (10 ml) for 10 min. The mixture was diluted with $CHCl_3$ (100 ml) and washed with H_2O (2 × 50 ml). The org. phase was dried (Na_2SO_4) and evaporated and the crude product purified by CC (silica gel, 10 × 2.5 cm, $CHCl_3$). Amorphous solid. UV (MeOH): 232 (4.32), 272 (4.36). ^{1}H-NMR ((D$_6$)DMSO): 8.65 (*s*, NH); 8.49, 8.25 (2*s*, H–C(2), H–C(8)); 8.07–7.11 (*m*, 15 arom. H); 6.54 (*d*, H–C(1')); 6.30 (*dd*, H–C(2')); 5.92 (*dd*, H–C(3')); 5.86 (*t*, OH–C(5')); 4.72 (*s*, PhCH_2); 4.55 (*m*, H–C(4')); 3.83 (*m*, 2 H–C(5')). Anal. calc. for $C_{31}H_{27}N_5O_6$ (565.5): C 65.83, H 4.81, N 12.38; found: C 65.91, H 4.85, N 12.29.

1-[5-O-(Monomethoxytrityl)-β-D-ribofuranosyl]-1H-1,2,4-triazole-3-carboxamide (**8**). A mixture of ribavirin (**7**; 1 g, 4.1 mmol) and 4-methoxytrityl chloride (1.5 g, 4.9 mmol) in pyridine (50 ml) was stirred at r.t. for 48 h, evaporated, and co-evaporated with toluene (2 × 30 ml). The residue was dissolved in $CHCl_3$ (100 ml) and washed with H_2O (2 × 50 ml). The org. layer was dried (Na_2SO_4), evaporated to a small volume (*ca.* 7 ml), and precipitated with hexane to give, after drying under high vacuum, 1.95 g (92%) of **8**. UV (MeOH): 231 (4.27). ^{1}H-NMR ((D$_6$)DMSO): 8.83 (*s*, H–C(5)); 7.75, 7.63 (2*s*, NH_2); 7.36–6.83 (*m*, 10 arom. H); 5.94 (*d*, H–C(1)); 5.65 (*d*, OH–C(2')); 5.20 (*d*, OH–C(3')); 4.20 (*m*, H–C(2')); 4.31 (*m*, H–C(3')); 4.07 (*m*, H–C(4')); 3.73 (*s*, MeO); 3.13 (*m*, 2 H–C(5')). Anal. calc. for $C_{28}H_{28}N_4O_6$ (516.5): C 65.10, H 5.46, N 10.84; found: C 65.30, H 5.30, N 10.79.

1-[2,3-Di-O-benzoyl-5-O-(monomethoxytrityl)-β-D-ribofuranosyl]-1H-1,2,4-triazole-3-carboxamide (**9**) *and 1-[3-O-Benzoyl-5-O-(monomethoxytrityl)-β-D-ribofuranosyl]-1H-1,2,4-triazole carboxamide* (**10**). To a soln. of **8** (1.85 g, 3.48 mmol) in MeCN (40 ml), Et_3N (6.3 ml), and DMAP (32 mg, 0.26 mmol), a soln. of benzoyl cyanide (0.55 g, 4.18 mmol) in MeCN (10 ml) was added dropwise within 3 h. The mixture was stirred at r.t. for 18 h and

evaporated. Purification by CC (silica gel, 20 × 3.5 cm, hexane/AcOEt 3:1 → AcOEt) gave, after drying under high vacuum, 0.4 g (16%) of **9** and 1.3 g (57%) of **10** as colorless foams.

9: UV (MeOH): 231 (4.63). ^{1}H-NMR ((D_6)DMSO): 8.94 (*s*, H–C(5)); 7.93–6.78 (*m*, 26 H, NH_2, arom. H); 6.65 (*d*, H–C(1′)); 6.10 (*dd*, H–C(2′)); 6.03 (*dd*, H–C(3′)); 4.60 (*m*, H–C(4′)); 3.69 (*s*, MeO); 3.43 (*m*, 2 H–C(5′)). Anal. calc. for $C_{42}H_{36}N_4O_8$ (724.85): C 69.60, H 5.00, N 7.73; found: C 69.35, H 4.89, N 17.85.

10: UV (MeOH): 231 (4.47). ^{1}H-NMR ((D_6)DMSO): 8.93 (*s*, H–C(5)); 8.04–6.81 (*m*, 21 H, NH_2, arom. H); 6.12 (*d*, OH–C(2′)); 6.09 (*d*, H–C(1′)); 5.54 (*dd*, H–C(3′)); 4.90 (*dd*, H–C(2′)); 4.43 (*m*, H–C(4′)); 3.70 (*s*, MeO); 3.30 (*m*, 2 H–C(5′)). Anal. calc. for $C_{35}H_{32}N_4O_7$ (620.7): C 67.73, H 5.19, N 9.02; found: C 67.48, H 5.10, N 8.94.

*1-(2,3-Di-*O*-benzoyl-β-*D*-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide* (**11**). A soln. of **9** (0.36 g, 0.5 mmol) in 80% AcOH (30 ml) was stirred at 50° for 15 min and evaporated. The residue was co-evaporated with EtOH (2 × 30 ml) and crystallized from EtOH: 176 mg (78%) of **11**. M.p. 172–173°. UV (MeOH): 229 (4.54). ^{1}H-NMR ((D_6)DMSO): 8.95 (*s*, H–C(5)); 7.95–7.40 (*m*, 12 H, NH_2, arom. H); 6.57 (*d*, H–C(1′)); 6.05 (*dd*, H–C(2′)); 5.87 (*dd*, H–C(3′)); 4.55 (*dd*, H–C(4′)); 3.75 (*m*, 2 H–C(5′)). Anal. calc. for $C_{22}H_{20}N_4O_7$ (452.4): C 58.40, H 4.45, N 12.38; found: C 58.20, H 4.32, N 12.27.

3′-O-Benzoyl-N^6-benzyl-5′-O-(monomethoxytrityl)adenosine 2-[2-(4-Nitrophenyl)ethyl Triethylammonium Phosphate] (**13**). To a soln. of 1*H*-1,2,4-triazole (92 mg, 1.33 mmol) in pyridine (1.3 ml), 2-chlorophenyl phosphorodichloridate (160 mg, 0.65 mmol) was added. After stirring at r.t. for 10 min, the mixture was cooled with ice, and a soln. of **5** (0.32 g, 0.44 mmol) in pyridine (0.9 ml) was added. After 3 h, 2-(4-nitrophenyl)ethanol (0.54 g, 3.25 mmol) was added and the mixture stirred at r.t. for 18 h, diluted with $CHCl_3$ (100 ml), and washed with 0.05M $(Et_3NH)HCO_3$ (2 × 50 ml). The org. phase was dried (Na_2SO_4), evaporated, and co-evaporated with toluene (2 × 20 ml). The residue was dissolved in a soln. of 4-nitrobenzaldoxim (0.72 g, 4.33 mmol) in dioxane/Et_3N/H_2O 1:1:1 (30 ml). After stirring at 4° for 20 h, the mixture was evaporated and the residue purified by CC (silica gel, 10 × 2.5 cm, $CHCl_3$/MeOH/Et_3N 95:4:1): 0.38 g (82%) of **13**. Colorless foam. UV (MeOH): 233 (4.47), 272 (4.46). ^{1}H-NMR ((D_6)DMSO): 8.49 (*s*, NH); 8.32, 8.13 (2*s*, H–C(2), H–C(8)); 8.03–6.79 (*m*, 28 arom. H); 6.27 (*d*, H–C(1′)); 5.86 (*dd*, H–C(3′)); 5.65 (*m*, H–C(2′)); 4.70 (*s*, PhCH_2); 4.39 (*m*, H–C(4′)); 3.68 (*s*, MeO). Anal. calc. for $C_{58}H_{62}N_7O_{11}P$ (1064.1): C 65.46, H 5.87, N 9.12; found: C 65.58, H 5.78, N 8.97.

*1-[3-O-Benzoyl-5-O-(monomethoxytrityl)-β-*D*-ribofuranosyl]-1*H*-1,2,4-triazole-3-carboxamide 2′-[2-(4-Nitrophenyl)ethyl Triethylammonium Phosphate]* (**14**). To a mixture of 1*H*-1,2,4-triazole (0.16 g, 2.38 mmol) and 2-chlorophenyl phosphorodichloridate (0.27 g, 1.19 mmol) in pyridine (2.2 ml), a soln. of **10** (0.5 g, 0.81 mmol) in pyridine was added dropwise for 15 min at + 4°. After 3 h stirring at r.t., 2-(4-nitrophenyl)ethanol (0.54 g, 3.23 mmol) was aded. The mixture was stirred at r.t. for 18 h, diluted with $CHCl_3$ (200 ml), and washed with 0.05M $(Et_3NH)HCO_3$ (2 × 100 ml). The org. phase was dried (Na_2SO_4), evaporated, and co-evaporated with toluene (2 × 50 ml). The residue was dissolved in a soln. of 4-nitrobenzaldoxime (0.45 g, 2.71 mmol) in dioxane/Et_3N/H_2O 1:1:1 (18 ml). The mixture was stirred at r.t. for 24 h, evaporated, and co-evaporated with toluene (2 × 20 ml). The residue was purified by CC (silica gel, 10 × 2.5 cm, $CHCl_3$, and then $CHCl_3$/MeOH/Et_3N 95:4:1): 0.37 g (48%) of **14**. Colorless foam. UV (MeOH): 230 (4.39), 273 (4.07). ^{1}H-NMR ((D_6)DMSO): 8.94 (*s*, H–C(5)); 8.05–6.75 (*m*, 25 H, NH_2, arom. H); 6.30 (*d*, H–C(1′)); 5.78 (*dd*, H–C(3′)); 5.61 (*m*, H–C(2′)); 4.45 (*m*, H–C(4′)); 4.25 (*m*, OCH_2CH_2Ph); 3.69 (*s*, MeO); 3.31 (*m*, 2 H–C(5′)); 2.81 (*m*, OCH_2CH_2Ph). Anal. calc. for $C_{49}H_{55}N_6O_{12}P$ (951.0): C 61.88, H 5.82, N 8.83; found: C 61.71, H 5.70, N 8.69.

N^6,3′-O-Dibenzoyladenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]} → 5′}-2′,3′-di-O-benzoyl-N^6-benzyladenosine (**16**). A mixture of **6** (56 mg, 0.1 mmol), **15** (151 mg, 0.14 mmol), 1*H*-tetrazole (59 mg, 0.84 mmol), and TpsCl (85 mg, 0.28 mmol) in pyridine (1 ml) was stirred at r.t. for 16 h, diluted with $CHCl_3$ (50 ml), and washed with 0.05M $(Et_3NH)HCO_3$ (2 × 15 ml). The org. phase was dried (Na_2SO_4), evaporated, and co-evaporated with toluene (2 × 15 ml). The residue was dissolved in 2% TsOH soln. (10 ml), and after 10 min, diluted with $CHCl_3$ (50 m), and washed with 0.05M $(Et_3NH)HCO_3$. The org. layer was dried (Na_2SO_4) and evaporated. The residue was purified by CC (silica gel, 10 × 2.5 cm, $CHCl_3$): 105 mg (85%) of **16**. Colorless foam. UV (MeOH): 234 (4.70), 272 (4.66). Anal. calc. for $C_{63}H_{54}N_{11}O_{16}P$ (1252.2): C 60.43, H 4.34, N 12.30; found: C 60.59, H 4.42, N 12.18.

3′-O-Benzoyl-N^6-benzyladenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]} → 5′}-2′,3′-di-O-benzoyl-N^6-benzyladenosine (**17**). As described for **16**, with **6** (40 mg, 0.071 mmol), **13** (105 mg, 0.1 mmol), pyridine (0.7 ml), TpsCl (60 mg, 0.188 mmol), 1*H*-tetrazole (42 mg, 0.59 mmol), 2% TsOH soln. (5.7 ml), and 0.05M $(Et_3NH)HCO_3$. CC (silica gel, 10 × 2.5 cm, $CHCl_3$) gave 71 mg (81%) of **17**. Colorless foam. UV (MeOH): 234 (4.71), 272 (4.65). Anal. calc. for $C_{63}H_{56}N_{11}O_{15}P$ (1238.2): C 61.11, H 4.55, N 12.44; found: C 61.30, H 4.60, N 12.23.

3′-O-Benzoyl-N^6-benzyladenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]} → 5′}-N^6,2′-O,3′-O-tribenzoyladenosine (**18**). As described for **16**, with **12** (40 mg, 0.069 mmol), **13** (102 mg, 0.096 mmol), pyridine (0.7 ml), TpsCl (60 mg, 0.188 mmol), 1*H*-tetrazole (42 mg, 0.59 mmol), 2% TsOH soln. (5.5 ml), and 0.05M $(Et_3NH)HCO_3$. CC (silica gel,

10 × 2.5 cm, $CHCl_3$) gave 75 mg (87%) of **18**. Colorless foam. UV (MeOH): 234 (4.70), 272 (4.66). Anal. calc. for $C_{63}H_{54}N_{11}O_{16}P$ (1252.2): C 60.43, H 4.34, N 12.30; found: C 60.55, H 4.29, N 12.19.

N^6,3′-O-Dibenzoyladenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]} → 5′}-1-(2,3-di-O-benzoyl-β-D-ribofuranosyl)-1H-1,2,4-triazole-3-carboxamide (**19**). As described for **16**, with **11** (70 mg, 0.15 mmol), **15** (0.2 g, 0.18 mmol), pyridine (2 ml), TpsCl (0.17 g, 0.56 mmol), 1*H*-tetrazole (80 mg, 1.14 mmol), 2% TsOH soln. (10 ml), and 0.05M $(Et_3NH)HCO_3$. CC (silica gel, 10 × 2.5 cm, $CHCl_3$ → $CHCl_3$/MeOH 19:1) gave 0.13 g (74%) of **19**. Colorless foam. UV (MeOH): 233 (4.72), 272 (4.35). Anal. calc. for $C_{54}H_{47}N_{10}O_{17}P$ (1139.0): C 56.94, H 4.15, N 12.29; found: C 57.07, H 4.21, N 12.14.

[1-(3-O-Benzoyl-β-D-ribofuranosyl)-1H-1,2,4-triazole-3-carboxamide]yl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]} →5′}-N^6,2′-O,3′-O-tribenzoyladenosine (**20**). As described for **16**, with **12** (58 mg, 0.1 mmol), **14** (144 mg, 0.15 mmol), pyridine (2 ml), TpsCl (91 mg, 0.3 mmol), 1*H*-tetrazole (63 mg, 0.9 mmol), 2% TsOH soln. (10 ml), and 0.05M $(Et_3NH)HCO_3$. CC (silica gel, 9 × 2.5 cm, $CHCl_3$ → $CHCl_3$/MeOH 19:1) gave 86 mg (76%) of **20**. Colorless foam. UV (MeOH): 233 (4.71), 273 (4.36). Anal. calc. for $C_{54}H_{47}N_{10}O_{17}P$ (1139.0): C 56.94, H 4.15, N 12.19; found: C 57.11, H 4.14, N 12.20.

Adenylyl-(2′–5′)-adenylyl-(2′–5′)-N^6-benzyladenosine Bis(triethylammonium) Salt (**22**). A mixture of **15** (127 mg, 0.12 mmol) and **16** (105 mg, 0.08 mmol) in pyridine (0.8 ml), in the presence of TpsCl (71 mg, 0.23 mmol) and 1*H*-tetrazole (49 mg, 0.7 mmol), was stirred at r.t. for 18 h, diluted with $CHCl_3$ (50 ml), and washed with 0.05M $(Et_3NH)HCO_3$ (2 × 20 ml). The org. layer was dried (Na_2SO_4), evaporated, and co-evaporated with toluene (2 × 10 ml). The residue was treated with 2% TsOH soln. (8 ml), stirred for 10 min, diluted with $CHCl_3$ (50 ml), and washed with 0.05M $(Et_3NH)HCO_3$ (2 × 15 ml). The org. layer was dried (Na_2SO_4) and evaporated. The residue was dissolved in 0.5M DBU in pyridine (16.4 ml) and stirred at r.t. for 18 h. Then the soln. was neutralized with 1M AcOH in pyridine (8.2 ml) and evaporated. The residue was dissolved in sat. NH_3/MeOH (40 ml), stirred at r.t. for 18 h, and evaporated, and the residue taken up in $CHCl_3/H_2O$ 1:1 (100 ml). The org. phase was applied to an ion-exchange *DEAE-Servacel-23-SS* column (20 × 1.5 cm, linear gradient of 0.005 → 0.2M $(Et_3NH)HCO_3$ buffer (pH 7.5). The product fractions were evaporated, and co-evaporated with MeOH (3 × 30 ml). The residual Et_3NH^+ salt was lyophilized (H_2O): 54 mg (53%) of **22**. UV (H_2O): 263 (4.52). ^{1}H-NMR (D_2O, *t*-BuOH as internal standard): 6.91, 6.83, 6.73, 6.71, 6.62, 6.51 (6*s*, H–C(2), H–C(8)); 6.13 (*m*, 5 arom. H); 4.80, 4.67, 4.60 (3*d*, 3 H–C(1′)).

Adenylyl-(2′–5′)-N^6-benzyladenylyl-(2′–5′)-adenosine Bis(triethylammonium) Salt (**23**). As described for **22**, with **15** (91 mg, 0.08 mmol), **18** (75 mg, 0.06 mmol), pyridine (0.6 ml), TpsCl (51 mg, 0.17 mmol), 1*H*-tetrazole (35 mg, 0.45 mmol), 2% TsOH soln. (5 ml), 0.5M DBU in pyridine (13.2 ml), 1M AcOH in pyridine (6.6 ml), and sat. NH_3 in MeOH (15 ml). Treatment with $CHCl_3/H_2O$ 1:1 (100 ml) gave, after ion exchange (*DEAE-Servacel 23-SS*), 49 mg (67%) of **23**. UV (H_2O): 263 (4.52). ^{1}H-NMR (D_2O, *t*-BuOH as internal standard): 6.86, 6.81 (2 H), 6.77, 6.56, 6.39 (5*s*, H–C(2), H–C(8)); 6.08 (*m*, 5 arom. H); 4.77, 4.73, 4.57 (3*d*, 3 H–C(1′)).

N^6-Benzyladenylyl-(2′–5′)-adenylyl-(2′–5′)-adenosine Bis(triethylammonium) Salt (**24**). As described for **22**, with **13** (32 mg, 0.03 mmol), **21** (32 mg, 0.025 mol), pyridine (0.3 ml), TpsCl (18 mg, 0.06 mmol), 1*H*-tetrazole (17 mg, 0.24 mmol), 2% TsOH soln. (3 ml), 0.5M DBU in pyridne (3.2 ml), 1M AcOH in pyridine (1.6 ml), and sat. NH_3 in MeOH (8 ml). Treatment with $CHCl_3/H_2O$ 1:1 (80 ml) gave, after ion exchange (*DEAE-Servacel 23-SS*), 16 mg (52%) of **24**. UV (H_2O): 263 (4.48). ^{1}H-NMR (D_2O, *t*-BuOH as internal standard): 6.92, 6.85, 6.73, 6.67 (2 H), 6.50, (5*s*, H–C(2), H–C(8)); 6.07 (*m*, 5 arom. H); 4.85, 4.67, 4.59 (3*d*, 3 H–C(1′)).

N^6-Benzyladenylyl-(2′–5′)-N^6-benzyladenylyl-(2′–5′)-N^6-benzyladenosine Bis(triethylammonium) Salt (**25**). As described for **22**, with **13** (85 mg, 0.08 mmol), **17** (71 mg, 0.06 mmol), pyridine (0.6 ml), TpsCl (48 mg, 0.16 mmol), 1*H*-tetrazole (34 mg, 0.48 mmol), 2% TsOH soln. (4.5 ml), 0.5M DBU in pyridine (9 ml), 1M AcOH in pyridine (4.5 ml), and sat. NH_3 in MeOH (30 ml). Treatment with $CHCl_3/H_2O$ 1:1 (100 ml) gave, after ion exchange (*DEAE-Servacel 23-SS*), 27 mg (76%) of **25**. UV (H_2O): 270 (4.72). ^{1}H-NMR (D_2O, *t*-BuOH as internal standard): 6.88, 6.83, 6.79, 6.73, 6.57, 6.50 (6*s*, H–C(2), H–C(8)); 6.04 (*m*, 15 arom. H); 4.82, 4.76, 4.62 (3*d*, 3 H–C(1′)).

Adenylyl-(2′–5′)-adenylyl-(2′–5′)-1-(β-D-ribofuranosyl)-1H-1,2,4-triazole-3-carboxamide Bis(triethylammonium) Salt (**26**). As described for **22**, with **15** (110 mg, 0.1 mmol), **19** (95 mg, 0.08 mmol), pyridine (0.9 ml), TpsCl (90 mg, 0.3 mmol), 1*H*-tetrazole (42 mg, 0.59 mmol), 2% TsOH soln. (5 ml), 0.5M DBU in pyridine (10 ml), 1M AcOH in pyridine (5 ml), and sat. NH_3 in MeOH (30 ml). Treatment with $CHCl_3/H_2O$ 1:1 (100 ml) gave, after ion exchange (*DEAE-Servacel 23-SS*), 31 mg (34%) of **26**. UV (H_2O): 260 (4.42). ^{1}H-NMR (D_2O, *t*-BuOH as internal standard): 7.13, 6.88, 6.83, 6.73, 6.45 (5*s*, H–C(2), H–C(5), H–C(8)); 4.85, 4.68, 4.50 (3*d*, 3 H–C(1′)).

Adenylyl-(2′–5′)-[1-(β-D-ribofuranosyl)-1H-1,2,4-triazole-3-carboxamide]yl-(2′–5′)-adenosine Bis(triethylammonium) Salt (**27**). As described for **22**, with **15** (86 mg, 0.08 mmol), **20** (57 mg, 0.05 mmol), pyridine (0.7 ml), TpsCl (73 mg, 0.24 mmol), 1*H*-tetrazole (50 mg, 0.72 mmol), 2% TsOH soln. (4 ml), 0.5M DBU in pyridine (7 ml),

1M AcOH in pyridine (3.5 ml), and sat. NH_3 in MeOH (25 ml). Treatment with $CHCl_3/H_2O$ 1:1 (80 ml) gave, after ion exchange (*DEAE-Servacel 23-SS*), 13 mg (24%) of **27**. UV (H_2O): 260 (4.42). ^{1}H-NMR (D_2O, *t*-BuOH as internal standard): 7.06, 6.98, 6.90, 6.80, 6.71 (5*s*, H–C(2), H–C(5), H–C(8)); 4.87, 4.77, 4.62 (3*d*, 3 H–C(1')).

*1-[(β-*D*-Ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide]yl-(2'–5')-adenylyl-(2'–5')-adenosine Bis(triethylammonium) Salt* (**28**). As described for **22**, with **14** (76 mg, 0.08 mmol), **21** (63 mg, 0.05 mmol), pyridine (0.8 ml), TpsCl (73 mg, 0.24 mmol), 1*H*-tetrazole (50 mg, 0.72 mmol), 2% TsOH soln. (5 ml), 0.5M DBU in pyridine (8 ml), 1M AcOH in pyridine (4 ml), and sat. NH_3 in MeOH (30 ml). Treatment with $CHCl_3/H_2O$ 1:1 (100 ml) gave, after ion exchange (*DEAE-Servacel 23-SS*), 26 mg (48%) of **28**. UV (H_2O): 260 (4.43). ^{1}H-NMR (D_2O, *t*-BuOH as internal standard): 7.06, 6.88 (2 H), 6.75, 6.65 (4*s*, H–C(2), H–C(5), H–C(8)); 4.84, 4.73, 4.59 (3*d*, 3 H–C(1')).

REFERENCES

[1] C. Hörndler, R. J. Suhadolnik, N. F. Muto, E. E. Henderson, M.-X. Guang, W. Pfleiderer, *Helv. Chim. Acta* **1997**, *80*, 767.

[2] A. G. Hovanessian, R. E. Brown, J. M. Kerr, *Nature (London)* **1977**, *268*, 537.

[3] P. F. Torrence, in 'Biological Response Modifiers. New Approaches to Disease Intervention', Academic Press, Orlando 1985, p. 77.

[4] A. Kimchi, H. Shure, M. Revel, *Nature (London)* **1979**, *282*, 727.

[5] Y. Devash, A. Gera, D. H. Willis, M. Reichman, W. Pfleiderer, R. Charubala, I. Sela, R. J. Suhadolnik, *J. Biol. Chem.* **1984**, *259*, 3482.

[6] S. N. Mikhailov, W. Pfleiderer, *Tetrahedron Lett.* **1985**, *26*, 2059.

[7] P. Herdewijn, R. Charubala, R. Pauwels, E. De Clercq, W. Pfleiderer, *Nucleosides Nucleotides* **1987**, *6*, 443.

[8] K. Kariko, R. W. Sobol, L. Suhadolnik, S. W. Li, N. L. Reichenbach, R. J. Suhadolnik, W. Pfleiderer, *Biochemistry* **1987**, *26*, 7127.

[9] P. Herdewijn, R. Charubala, W. Pfleiderer, *Helv. Chim. Acta* **1989**, *72*, 1729.

[10] R. Charubala, W. Pfleiderer, R. W. Sobol, S. W. L., R. J. Suhadolnik, *Helv. Chim. Acta* **1989**, *72*, 1354.

[11] Y. Kitade, Y. Nakata, K. Hirota, Y. Maki, A. Pabuccuoglu, P. F. Torrence, *Nucleic Acids Res.* **1991**, *19*, 4103.

[12] W. Pfleiderer, F. Himmelsbach, R. Charubala, *Bioorg. Med. Chem. Lett.* **1994**, *8*, 1047.

[13] E. I. Kvasyuk, T. I. Kulak, I. A. Mikhailopulo, R. Charubala, W. Pfleiderer, *Helv. Chim. Acta* **1995**, *78*, 1777.

[14] W. E. G. Müller, B. E. Weiler, R. Charubala, W. Pfleiderer, L. Leserman, R. W. Sobol, R. J. Suhadolnik, H. C. Schröder, *Biochemistry* **1991**, *30*, 2027.

[15] H. C. Schröder, R. J. Suhadolnik, W. Pfleiderer, R. Charubala, W. E. G. Müller, *Int. J. Biochem.* **1992**, *24*, 55.

[16] R. Charubala, W. Pfleiderer, in 'Progress in Molecular and Subcellular Biology', Eds. W. E. G. Müller and H. C. Schröder, Springer Verlag, Heidelberg, 1994, vol. 14, p. 114.

[17] M. Wasner, E. E. Henderson, R. J. Suhadolnik, W. Pfleiderer, *Helv. Chim. Acta* **1994**, *77*, 1757.

[18] M. Wasner, R. J. Suhadolnik, S. E. Horvath, M. E. Adelson, N. Kon, M.-X. Guang, E. E. Henderson, W. Pfleiderer, *Helv. Chim. Acta* **1996**, *79*, 619.

[19] E. I. Kvasyuk, T. I. Kulak, N. B. Khripach, I. A. Mikhailopulo, E. Uhlmann, R. Charubala, W. Pfleiderer, *Synthesis* **1987**, 535.

[20] A. I. Zinchenko, V. N. Barai, V. I. Lyachovez, E. I. Kvasyuk, I. A. Mikhailopulo, *Bioorg. Khim. (Rus.)* **1988**, *14*, 1401.

[21] E. I. Kvasyuk, E. N. Kalinichenko, T. I. Kulak, T. L. Podkopaeva, I. A. Mikhailopulo, I. L. Popov, V. N. Barai, A. I. Zinchenko, *Bioorg. Khim. (Rus.)* **1985**, *11*, 1239.

[22] E. I. Kvasyuk, T. I. Kulak, G. V. Zaitseva, I. A. Mikhailopulo, *Bioorg. Khim. (Rus.)* **1984**, *10*, 506.

[23] M. Wasner, R. J. Suhadolnik, S. E. Horvath, M. E. Adelson, N. Kon, M.-X. Guang, E. E. Henderson, W. Pfleiderer, *Helv. Chim. Acta* **1996**, *79*, 609.