2. Nucleotides

Part XL[1])

Synthesis and Characterization of Modified 2′–5′ Adenylate Trimers – Potential Antiviral Agents

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(1.IX.93)

2′–5′ Adenylate trimers **41**–**44** carrying the (*tert*-butyl)dimethylsilyl (tbds) group at the 3′-OH position of various sugar moieties were synthesized *via* the phosphoramidite method. The use of the (*tert*-butyloxy)carbonyl (boc) and 2-(4-nitrophenyl)ethylsulfonyl (npes) groups for 2′-OH protection in neighbourhood to the 3′-*O*-tbds residue was compared during the synthesis of the target trimers. For other functional positions, the use of the 2-(4-nitrophenyl)ethyl (npe) and 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) blocking groups were favoured.

1. Introduction. – The establishment of the 2′–5′ A synthetase/RNase L system as a part of the interferon-induced antiviral mechanism in mammalian cells may also play a role in regulation of cell growth and differentiation [2] [3]. The 2′–5′ A synthetase is an allosterically regulated enzyme [4], activated by dsRNA, which polymerizes ATP to give (2′–5′)poly[A], a (2′–5′)-linked oligoribonucleotide, of which the trimer showed so far the highest activity in subsequent activation of the target enzyme RNase L. RNase L, the activated endoribonuclease, cleaves then messenger and ribosomal RNA's [5], resulting in inhibition of translation. To explore the biological role of the 2′–5′ A synthetase/RNase L system, several attempts were undertaken [6–8] or are in progress to synthesize by chemical means structurally modified 2′–5′ A molecules with modifications in the aglycon and sugar moiety, respectively. Since the presence of a 3′-*O*-methyl group at the 2′-terminal end of (2′–5′)ApApA induced a higher biological index, obviously due to greater enzymatic stability [9], we decided to synthesize some additional (2′–5′)ApApA analogous in which, *e.g.*, the 2′-terminal adenosine moiety is 3′-*O*-substituted with the [(*tert*-butyl)dimethylsilyl] (tbds) group. Furthermore, the findings [10–12] of different functions of the three 3′-OH groups of (2′–5′)ApApA showing that only the penultimate 3′-OH group is essential for the activation of RNase L forced us to synthesize the analogous (2′–5′)A(tbds)$^{3'}$pApA(tbds)$^{3'}$ · 2 Et_3N (**41**) and (2′–5′)$A_{d3'}$pApA(tbds)$^{3'}$ · 2 Et_3N (**43**).

2. Syntheses. – The chemical solution syntheses of the 2′–5′ A trimers carrying one or two 3′-*O*-tbds groups at the 2′- and 5′-terminal ends (see **43** and **41**, resp.) were achieved by the phosphoramidite approach and final deprotection of all protecting

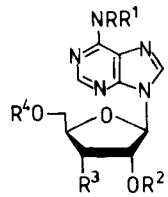
[1]) Part XXXIX: [1].

groups except the tbds groups. To achieve this goal, our general strategy was based upon groups to be removed by β-elimination such as the 2-(4-nitrophenyl)ethyl (npe) and 2-cyanoethyl (ce) groups for phosphate protection and the 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) group to block the N^6-amino function of adenosine [13]. Two alternative protecting groups for the 2′-OH position of the 2′-terminal end, neighbouring the 3′-*O*-silyl group, was envisaged by synthesizing the desired partially silylated 2′–5′ A trimers containing either the base-labile 2-(4-nitrophenyl)ethylsulfonyl (npes) [14] [15] or the acid-labile (*tert*-butyloxy)carbonyl (boc) groups [16] [17], respectively.

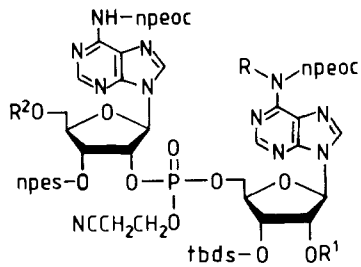
N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**1**) [13] [18] was chosen as starting material for monomethoxytritylation at the 5′-*O*-position which proceeded almost quantitatively to 5′-*O*-(monomethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**2**) [18]. Reaction of **2** with (*tert*-butyl)chlorodimethylsilane in pyridine and in presence of 1*H*-imidazole led to the corresponding silylated compounds **3** (32%), **4** (35%), and **5** (21%) after chromatographic separation. The desired 3′-*O*-silylated **4** was enriched by the method of *Sung* and *Narang* [19] *via* partial isomerization of the 2′-*O*-tbds group of **3** in MeOH/Et_3N. Then, the building block **4** was used on one hand for the reaction with di(*tert*-butyl) dicarbonate [16] [17] in pyridine and in presence of 4-(dimethylamino)-pyridine to form the fully protected adenosine derivative **6** in 91% yield. Subsequent acid-catalysed detritylation with 3% CCl_3COOH in CH_2Cl_2 afforded the N^6,2′-*O*-bis-[(*tert*-butyl)oxycarbonyl] derivative **7** in good yield, *i.e.* without harming the boc groups [17]. On the other hand, **4** was reacted with 2-(4-nitrophenyl)ethylsulfonyl chloride [14] [15] in pyridine to give the 2′-*O*-[2-(4-nitrophenyl)ethylsulfonyl] derivative **8** which led, on detritylation with 2% TsOH in CH_2Cl_2/MeOH 4:1, to **9**.

Some of these intermediates were subject to various deprotection conditions to prove their utilization in the synthetic approach. Thus, DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) treatment of **7** led to compound **10** in good yield, as expected by cleavage of the N^6-[2-(4-nitrophenyl)ethoxycarbonyl] group *via* a β-elimination mechanism. Otherwise, deblocking of the boc groups of **7** was achieved by CF_3COOH [20] to give **11** without any migration of the tbds group. The same compound (TLC, spectra) was obtained on detritylation of **4**. For further comparisons, the isomeric 2′-*O*-silyl derivatives **12** was isolated from detritylation of **3**. However, 3′- to 2′-*O*-silyl migration was observed on treatment of compound **4** with DBU during the β-elimination of the N^6-[2-(4-nitrophenyl)ethoxycarbonyl] group in MeCN, leading to an isomer mixture of 2′-*O*-tbds and 3′-*O*-tbds derivatives **13** and **14**, respectively, in a molar ratio of *ca.* 1:1. DBU Treatment of **8** verified the fact of 3′- to 2′-*O*-isomerization of the tbds group and led again to the isomer mixture **13**/**14**. Under these aspects, the boc protecting group seemed to be a better choice for blocking the 2′-OH position neighbouring a *O*-tbds group.

Additional monomeric blocks for the middle part and the 5′-terminal end of the anticipated A trimers **41** and **43** were the 3′-deoxy-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**16**; obtained from **15**) [21], its 5′-*O*-monomethoxytrityl derivative **17** [21] and, obtained from **1** [13] [18], also the 5′-*O*-monomethoxytrityl (**2**) [18] and the 5′-*O*-dimethoxytrityl compound **18**. Silylation of **18** with (*tert*-butyl)chlorodimethylsilane in pyridine and in presence of 1*H*-imidazole gave again a mixture of the 2′-*O*-silyl, 3′-*O*-silyl, and 2′,3′-bis-*O*-silyl derivatives **19**–**21** in 33, 50, and 8% yield, respectively [22]. Likewise, treatment of **2** with 2-(4-nitrophenyl)ethylsulfonyl chloride [14] [15] in pyridine led also to a mixture of three compounds, from which the 3′-*O*-npes derivative **22** [23]

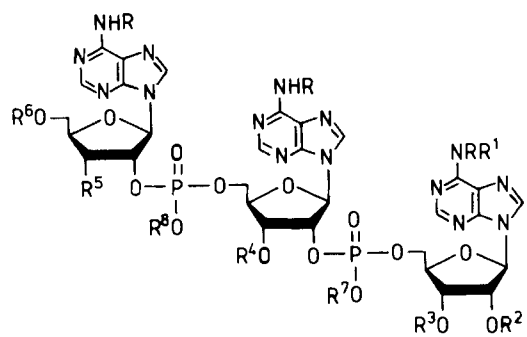


	R	R^1	R^2	R^3	R^4
1	npeoc	H	H	OH	H
2	npeoc	H	H	OH	MeOTr
3	npeoc	H	tbds	OH	MeOTr
4	npeoc	H	H	Otbds	MeOTr
5	npeoc	H	tbds	Otbds	MeOTr
6	npeoc	boc	boc	Otbds	MeOTr
7	npeoc	boc	boc	Otbds	H
8	npeoc	H	npes	Otbds	MeOTr
9	npeoc	H	npes	Otbds	H
10	H	boc	boc	Otbds	H
11	npeoc	H	H	Otbds	H
12	npeoc	H	tbds	OH	H
13	H	H	tbds	OH	MeOTr
14	H	H	H	Otbds	MeOTr
15	H	H	H	H	H
16	npeoc	H	H	H	H
17	npeoc	H	H	H	MeOTr
18	npeoc	H	H	OH	$(MeO)_2Tr$
19	npeoc	H	tbds	OH	$(MeO)_2Tr$
20	npeoc	H	H	Otbds	$(MeO)_2Tr$
21	npeoc	H	tbds	Otbds	$(MeO)_2Tr$
22	npeoc	H	H	Onpes	MeOTr
23	npeoc	H	tbds	Onpes	$(MeO)_2Tr$
24	npeoc	H	H	Onpes	$(MeO)_2Tr$



	R	R^1	R^2
31	boc	boc	$(MeO)_2Tr$
32	boc	boc	H
33	H	npes	MeOTr
34	H	npes	H

tbds = (*tert*-butyl)dimethylsilyl, boc = (*tert*-butyloxy)-carbonyl, ce = 2-cyanoethyl, MeOTr = monomethoxytrityl, $(MeO)_2Tr$ = dimethoxytrityl, npeoc = 2-(4-nitrophenyl)ethoxycarbonyl, npe = 2-(4-nitrophenyl)ethyl, npes = 2-(4-nitrophenyl)ethylsulfonyl

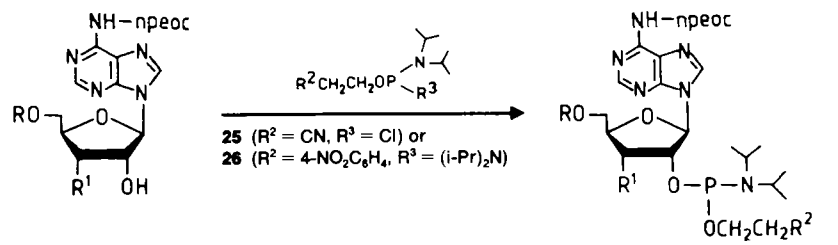


	R	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8
35	npeoc	boc	boc	tbds	npes	Otbds	MeOTr	ce	npe
36	npeoc	boc	boc	tbds	npes	H	MeOTr	ce	npe
37	npeoc	H	npes	tbds	npes	Otbds	MeOTr	ce	npe
38	npeoc	H	npes	tbds	npes	H	MeOTr	ce	npe
39	npeoc	H	npes	tbds	npes	Otbds	H	ce	npe
40	npeoc	H	npes	tbds	npes	H	H	ce	npe
41	H	H	H	tbds	H	Otbds	H	Et_3NH	Et_3NH
42	H	H	tbds	H	H	Otbds	H	Et_3NH	Et_3NH
43	H	H	H	tbds	H	H	H	Et_3NH	Et_3NH
44	H	H	tbds	H	H	H	H	Et_3NH	Et_3NH
45	npeoc	boc	boc	tbds	npes	Otbds	H	ce	npe

could be separated by tedious chromatographical separations in 30% yield. A better approach for the synthesis of the corresponding 5′-*O*-dimethoxytrityl-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]-3′-*O*-[2-(4-nitrophenyl)ethylsulfonyl)adenosine (**24**) was achieved from **19** by treatment with 2-(4-nitrophenyl)ethylsulfonyl chloride/pyridine at room temperature (→ **23**, 98% yield) followed by cleavage of the 2′-*O*-silyl group with 0.5M Bu_4NF (55% yield). The reaction was accompanied by formation of **18** in 28% yield resulting from the simultaneous removal of the npes group at the 3′-*O* position of **23**.

The synthesis of the various phosphoramidites was performed using two different reagents, chloro(2-cyanoethoxy)(diisopropylamino)phosphane (**25**) [24] for the 3′-*O*-npes adenosine derivatives and bis(diisopropylamino)[2-(4-nitrophenyl)ethoxy]phosphane (**26**) derived from PCl_3 and 2-(4-nitrophenyl)ethanol [25] for the 3′-*O*-tbds-adenosine or 3′-deoxyadenosine derivatives (*Scheme*). Thus, using **26** in presence of 1*H*-tetrazole, 3′-*O*-tbds-adenosine **4** and 3′-deoxyadenosine **17** [21] were converted into the corresponding phosphoramidites **27** and **28** in 92 and 89% yield, respectively. Also the phosphoramidites **29** and **30** were formed in excellent yield from the 3′-*O*-npes-adenosines **22** and **24**, respectively, using **25** in presence of *N,N*-diisopropylethylamine (*Hünig*'s base).

Scheme



	R	R¹
4	MeOTr	Otbds
17	MeOTr	H
22	MeOTr	Onpes
24	$(MeO)_2Tr$	Onpes

	R	R¹	R²
27	MeOTr	Otbds	4-$NO_2C_6H_4$
28	MeOTr	H	4-$NO_2C_6H_4$
29	MeOTr	Onpes	CN
30	$(MeO)_2Tr$	Onpes	CN

In the built-up of the modified 2′–5′ A trimers, the phosphoramidite **30** was condensed with **7** in presence of 1*H*-tetrazole in MeCN; subsequent oxidation with I_2/H_2O/pyridine gave the corresponding fully protected dimer **31** in only 62% yield due to incomplete reaction of **7**. Detritylation of **31** using 3% CCl_3COOH in CH_2Cl_2 afforded the 5′-OH dimer **32** after 8 min at 5° in 86% yield without harming the additional acid-labile boc groups. Analogous reaction of phosphoramidite **29** and **9** led to the fully protected dimer **33** in 84% yield (based on recovered **9**), and after detritylation to the 5′-OH dimer **34** in 88% yield after flash chromatography.

The syntheses of the fully protected trimers **35**–**38** were then separately performed from the 5′-OH dimers **32** and **34** and the phosphoramidites **27** and **28**, respectively, to give **35** in a non-optimized yield of 60%, but **36**–**38** in excellent yields.

The final removal of the various protecting groups of trimers **37** and **38**, except the tbds groups, were achieved by subsequent acid treatment (removal of 5′-*O*-MeOTr → **39**

and **40**, resp.) and DBU (1,8-diaza-bicyclo[5.4.0]undec-7-ene) treatment (removal of the ce, npe, npeoc, and npes groups by β-elimination). Under these basic conditions, the tbds group migrated partially in a known fashion to the neighbouring OH function in the ribose moiety leading to isomer mixtures of the 2′- and 3′-*O*-silylated oligomers, *i.e.* **41**/**42** (from **39**) and **43**/**44** (from **40**). Chromatography of these mixtures on a *DEAE-Sephadex* column using a linear gradient of $(Et_3NH)HCO_3$ buffer (pH 7.45) did not achieve separation but yielded, after lyophilization of the Et_3NH^+ salts, *ca.* 50% of **41**/**42** and **43**/**44**, respectively. HPLC Analysis (*Fig.*) and ^{1}H-NMR spectra indicated the presence of the 2′/3′-*O*-isomer mixtures.

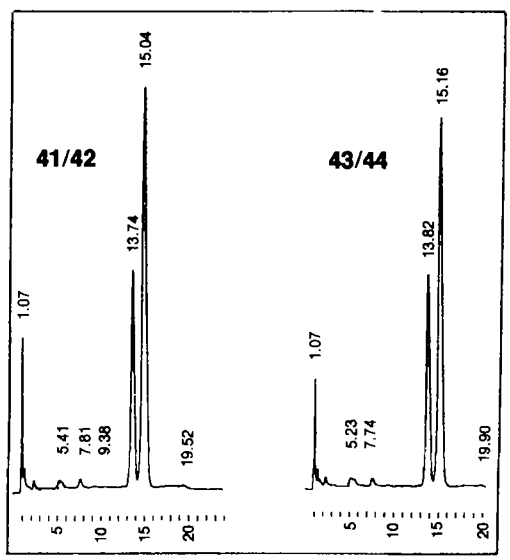


Figure. *HPLC Analysis of the mixtures* **41**, **42** *and* **43**, **44**. *RP-18*, for elution gradient, see *Exper. Part.*

Starting from the other fully protected trimers **35** and **36**, even more problems were encountered during the final deprotection steps to form the required partially silylated 2′–5′ A trimers **41** and **43**, respectively. Detritylation worked fine with 2% TsOH in CH_2Cl_2/MeOH 4:1 to give, in the case of **35**, **45** in 80% yield without any attack at the additional acid-labile boc residues. DBU Treatment in MeCN to split off groups by β-elimination worked also well, but after separation of the corresponding intermediates by *DEAE-Sephadex* column chromatography, the final boc deprotection step using CF_3COOH led unexpectedly to a complex mixture of mainly unidentified breakdown products from which the anticipated trimers **41** and **43**, respectively, could not be isolated.

We thank the *Bundesministerium für Forschung und Technologie* (grant No. II-054-88) and the *Fonds der Chemischen Industrie* for financial support.

Experimental Part

General. TLC: Precoated silica gel thin-layer sheets *F 1500 LS 254* from *Schleicher & Schüll* or *60 F_{254}* from *Merck*. Flash column chromatography (FC): silica gel *Baker* (30–60 μm), 0.2 bar. Ion-exchange chromatography: *Pharmacia* (detection at 258 nm; flow rate 2 ml/min (flow programming); paper speed 100 ml/cm), column *XK 16/70*, packed with *DEAE Sephadex A 25* (HCO_3^-; 60 × 1 cm; *Pharmacia*). HPLC: *Merck-Hitachi, L 6200 Intelligent* pump; *D 2000* chromato-integrator, detection at 260 nm (*Uvikon 730 S LC*, Fa. *Kontron*); column *RP 18* (*LiChrospher* 125 × 4 mm, 5 m, *Merck 50943*); flow rate 1 ml/min; elution: *A* = 0.1M (Et_3NH)OAc buffer (pH 7.2)/MeCN 1:1, *B* = 0.1M (Et_3NH)OAc buffer; gradient: within 0–10 min, *A*/*B* 1:1→6:4, then within 10–20 min, *A*/*B* 6:4. UV/VIS: *Perkin Elmer, Lambda 5*; λ_{max} in nm (lg ε). ^{1}H-NMR: *Bruker AC 250*; in ppm rel. to $CDCl_3$ ((D_6)DMSO) as internal standard. ^{31}P-NMR: *JEOL JM GX-400*; in ppm rel. to 85% H_3PO_4 soln.

1. *2′-O-[(*tert*-Butyl)dimethylsilyl]-5′-O-(monomethoxytrityl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**3**). *3′-O-[(*tert*-Butyl)dimethylsilyl]-5′-O-(monomethoxytrityl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-adenosine* (**4**), *and 2′,3′-Bis-O-[(*tert*-butyl)dimethylsilyl]-5′-O-(monomethoxytrityl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**5**). 1.1. In abs. pyridine (20 ml), **2** (13 g, 17.7 mmol) was dried by 2 co-evaporations. The residue was then dissolved in abs. pyridine (40 ml), (*t*-Bu)Me_2SiCl (4.4 g, 29.2 mmol) and 1*H*-imidazole (4.0 g, 58.7 mmol) were added and stirred at r.t. for 5 h. The mixture was diluted with CH_2Cl_2 (300 ml), washed with H_2O 3 × 200 ml), dried (Na_2SO_4), evaporated, co-evaporated with toluene (3 × 50 ml), and purified by FC (32 × 4.5 cm). Elution with toluene/AcOEt 5:1 (3 l), 4:1 (3.6 l), 3:1 (1.2 l), 2:1 (1.5 l), 1:1 (1.2 l), and toluene/AcOEt 1:2 (0.5 l) gave first 4.2 g (21%) of **5**, then 4.8 g (32%) of **3**, and finally 5.3 g (35%) of **4** as amorphous solids. Overall yield 88%.

3: UV (MeOH): 267 (4.47), 233 (4.32). ^{1}H-NMR ($CDCl_3$): 8.65 (*s*, *H*–C(8)); 8.27 (br. *s*, N*H*); 8.17 (*s*, *H*–C(2)); 8.16 (*d*, 2 H *o* to NO_2); 7.50–7.15 (*m*, 12 H of MeO*Tr*, 2 H *m* to NO_2); 6.81 (*d*, 2 H *o* to MeO); 6.06 (*d*, H–C(1′)); 5.00 (*t*, H–C(2′)); 4.51 (*t*, OCH_2CH$_2$); 4.35 (*m*, H–C(3′)); 4.28 (*m*, H–C(4′)); 3.77 (*s*, MeO); 3.60–3.34 (2*m*, 2 H–C(5′)); 3.12 (*t*, OCH$_2$CH_2); 2.72 (*d*, OH–C(3′)); 0.81 (*s*, *t*-BuSi); –0.03 (*s*, MeSi); –0.18 (*s*, MeSi). Anal. calc. for $C_{45}H_{50}N_6O_9$ Si (847.0): C 63.81, H 5.95, N 9.92; found: C 64.00, H 6.02, N 9.92.

4: UV (MeOH): 266 (4.46), 233 (4.31). ^{1}H-NMR ($CDCl_3$): 8.68 (*s*, H–C(8)); 8.50 (br. *s*, NH); 8.18 (*s*, H–C(2)); 8.13 (*d*, 2 H *o* to NO_2); 7.50–7.10 (*m*, 12 H of MeO*Tr*, 2 H *m* to NO_2); 6.79 (*d*, 2 H *o* to MeO); 6.02 (*d*, H–C(1′)); 4.77 (*m*, H–C(2′)); 4.59 (*t*, H–C(3′)); 4.50 (*t*, OCH_2CH$_2$); 4.19 (*m*, H–C(4′)); 3.76 (*s*, MeO); 3.52 (*m*, 1 H–C(5′)); 3.30–3.16 (*m*, OH–C(2′), 1 H–C(5′)); 3.12 (t, OCH$_2$CH_2); 0.88 (*s*, *t*-BuSi); 0.08 (*s*, MeSi); 0.00 (*s*, MeSi). Anal. calc. for $C_{45}H_{50}N_6O_9$Si (847.0): C 63.81, H 5.95, N 9.92; found: C 63.64, H 5.86, N 9.76.

5: UV (MeOH): 267 (4.47), 233 (4.31). ^{1}H-NMR ($CDCl_3$): 8.61 (*s*, H–C(8)); 8.16 (*s*, H–C(2)); 8.15 (*d*, 2 H *o* to NO_2); 8.09 (br. *s*, NH); 7.50–7.15 (*m*, 12 H of MeO*Tr*, 2 H *m* to NO_2); 6.81 (*d*, 2 H *o* to MeO); 5.99 (*d*, H–C(1′)); 4.88 (*t*, H–C(2′)); 4.50 (*t*, OCH_2CH$_2$); 4.23 (*m*, H–C(3′), H–C(4′)); 3.77 (*s*, MeO); 3.58, 3.31 (2*m*, 2 H–C(5′)); 3.12 (*t*, OCH$_2$CH_2); 0.85 (*s*, *t*-BuSi); 0.74 (*s*, *t*-BuSi); 0.05 (*s*, MeSi); –0.01 (*s*, MeSi); –0.08 (*s*, MeSi); –0.35 (*s*, MeSi). Anal. calc. for $C_{51}H_{64}N_6O_9Si_2$ (961.3): C 63.72, H 6.71, N 8.74; found: C 63.81, H 6.84, N 8.88.

1.2. Compound **3** (9 g, 10.63 mmol) was converted partially [19] to **4** by treatment in a soln. of MeOH (200 ml) and Et_3N (0.5 ml) at r.t. for 5 h. Evaporation, co-evaporation with MeOH (2 × 30 ml), and purification by FC (16 × 5 cm, packed and eluted with toluene/AcOEt 5:1 (1.5 l), then 4:1 (2.5 l), 3.5:1.5 (2 l), 3:2 (1 l), 1:1 (1 l), and 2:3 (1 l)) gave 3.1 g (34%) of **3**, 4.2 g (47%) of **4**, and 1.1 g (12%) of **3**/**4**. Overall yield 92%.

2. *3′-O-[(*tert*-Butyl)dimethylsilyl]-*N^6*,2′-O-bis[(*tert*-butyloxy)carbonyl]-5′-O-(monomethoxytrityl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**6**). In abs. pyridine (20 ml), **4** (1.21 g, 1.43 mmol) was dried by 3 co-evaporations. The residue was taken up in abs. pyridine (10 ml), then 4-(dimethylamino)pyridine (174 mg, 1.43 mmol) and di(*tert*-butyl) dicarbonate (778 mg, 3.56 mmol) were added. After stirring at r.t. for 30 min, CH_2Cl_2 (120 ml) was added and the mixture washed 2 times with sat. $NaHCO_3$ soln. (60 ml), dried (Na_2SO_4), evaporated, and co-evaporated with toluene (3 × 30 ml) to remove pyridine. The resulting foam was purified by FC (16 × 2.5 cm, CH_2Cl_2/MeOH 99:1 (250 ml) and 98:2 (700 ml)): 1.36 g (91%) of **6**. Amorphous solid. UV (MeOH): 267 (4.30), 231 (sh, 4.31). ^{1}H-NMR ($CDCl_3$): 8.78 (*s*, H–C(8)); 8.21 (*s*, H–C(2)); 8.02 (*d*, 2 H *o* to NO_2); 7.40–7.20 (*m*, 12 H of MeO*Tr*); 7.09 (*d*, 2 H *m* to NO_2); 6.78 (*d*, 2 H *o* to MeO); 6.27 (*d*, H–C(1′)); 5.85 (*t*, H–C(2′)); 4.91 (*t*, H–C(3′)); 4.34, 4.33 (2*t*, OCH_2CH$_2$); 4.26 (*m*, H–C(4′)); 3.76 (*s*, MeO); 3.53, 3.28 (2*m*, 2 H–C(5′)); 2.86 (*t*, OCH$_2$CH_2); 1.45 (*s*, *t*-Bu); 1.35 (*s*, *t*-Bu); 0.88 (*s*, *t*-BuSi); 0.11 (*s*, MeSi); 0.03 (*s*, MeSi). Anal. calc. for $C_{55}H_{66}N_6O_{13}$Si (1047.2): C 63.08, H 6.35, N 8.03; found: C 63.27, H 6.40, N 7.90.

3. *3′-O-[(*tert*-Butyl)dimethylsilyl]-*N^6*,2′-O-bis[(*tert*-butyloxy)carbonyl]-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**7**). A soln. (45 ml) of 3% CCl_3COOH in CH_2Cl_2 was stirred together with **6** (906 mg, 0.865 mmol) for 20 min at r.t. Then, the mixture was diluted with CH_2Cl_2 (100 ml), washed with sat. $NaHCO_3$ soln. (2 × 50 ml), dried ($MgSO_4$), and evaporated. Purification was achieved by FC (22 × 2.5 cm, toluene/AcOEt 4:1 (500 ml), 3:1

(400 ml), and 2:1 (400 ml)): 720 mg (72%) of **7**. Amorphous colourless solid. UV (MeOH): 267 (4.28). ^{1}H-NMR ($CDCl_3$): 8.85 (*s*, H–C(8)); 8.11 (*s*, H–C(2)); 8.06 (*d*, 2 H *o* to NO_2); 7.16 (*d*, 2 H *m* to NO_2); 6.12 (*d*, H–C(1′)); 5.60 (*m*, H–C(2′)); 4.81 (br. *s*, OH–C(5′)); 4.77 (*d*, H–C(3′)); 4.46 (*t*, OCH_2CH_2); 4.27 (*s*, H–C(4′)); 4.02, 3.79 (2*m*, 2 H–C(5′)); 3.00 (*t*, OCH_2CH_2); 1.42 (*s*, *t*-Bu); 1.39 (*s*, *t*-Bu); 0.95 (*s*, *t*-BuSi); 0.13 (*s*, Me_2Si). Anal. calc. for $C_{35}H_{50}N_6O_{12}Si$ (774.9): C 54.25, H 6.50, N 10.85; found: C 54.29, H 6.51, N 10.66.

4. *3′-O-[(*tert*-Butyl)dimethylsilyl]-5′-O-(monomethoxytrityl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-2′-O-[2-(4-nitrophenyl)ethylsulfonyl]adenosine* (**8**). In abs. pyridine (15 ml), **4** (1.24 g, 1.46 mmol) was dried by 3 co-evaporations. The residue was dissolved in abs. pyridine (15 ml), 2-(4-nitrophenyl) ethylsulfonyl chloride [14] [15] (730 mg, 2.92 mmol) added, and the mixture stirred at r.t. for 75 min, then evaporated to a small volume (4 ml), and co-evaporated with toluene (2 × 20 ml) to remove pyridine. FC (14 × 2.5 cm, toluene/AcOEt 3:1 (800 ml)) yielded 1.2 g (77%) of **8**. Amorphous solid. UV (MeOH): 272 (sh, 4.53), 267 (4.57), 234 (sh, 4.39). ^{1}H-NMR ($CDCl_3$): 8.56 (*s*, H–C(8)); 8.18–8.05 (*m*, H–C(2), NH; 4 H *o* to NO_2); 7.43–7.14 (*m*, 4 H *m* to NO_2, 12 H of MeO*Tr*); 6.78 (*d*, 2 H *o* to MeO); 6.26 (*d*, H–C(1′)); 5.88 (*t*, H–C(2′)); 4.73 (*t*, H–C(3′)); 4.52 (*t*, OCH_2CH_2); 4.19 (*m*, H–C(4′)); 3.76 (*s*, MeO); 3.55 (*m*, 1 H–C(5′)); 3.34–3.04 (*m*, $SO_2CH_2CH_2$, 1 H–C(5′)); 3.14 (*t*, OCH_2CH_2); 0.85 (*s*, *t*-BuSi); 0.11 (*s*, MeSi); 0.00 (*s*, MeSi). Anal. calc. for $C_{53}H_{57}N_7O_{13}SSi \cdot 1/2$ toluene (1106.3): C 61.34, H 5.56, N 8.86; found: C 61.13, H 5.44, N 8.51.

5. *3′-O-[(*tert*-Butyl)dimethylsilyl]-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-2′-O-[2-(4-nitrophenyl)ethylsulfonyl]adenosine* (**9**). A soln. of **8** (1.02 g, 0.96 mmol) in CH_2Cl_2/MeOH 4:1 (19 ml) containing 2% TsOH was kept at r.t. for 20 min. The mixture was diluted with AcOEt (100 ml), washed twice with H_2O (2 × 40 ml), dried (Na_2SO_4), evaporated, and submitted to FC (19 × 2.5 cm, toluene/AcOEt 1:1 (400 ml) and 1:2 (300 ml)): 550 mg (72%) of **9**. Solid foam. UV (MeOH): 296 (sh, 3.89), 271 (sh, 4.53), 267 (4.56). ^{1}H-NMR ($CDCl_3$): 8.72 (*s*, H–C(8)); 8.19–8.11 (*m*, 4 H *o* to NO_2, NH); 8.03 (*s*, H–C(2)); 7.42 (*d*, 2 H *m* to NO_2); 7.17 (*d*, 2 H *m* to NO_2); 6.12 (*d*, H–C(1′)); 5.79 (*m*, H–C(2′), OH–C(5′)); 4.68 (*d*, H–C(3′)); 4.53 (*t*, OCH_2CH_2); 4.24 (*s*, H–C(4′)); 3.94, 3.73 (2*m*, 2 H–C(5′)); 3.20–2.96 (*m*, OCH_2CH_2, $SO_2CH_2CH_2$); 0.94 (*s*, *t*-BuSi); 0.15 (*s*, MeSi); 0.14 (*s*, MeSi). Anal. calc. for $C_{33}H_{41}N_7O_{12}SSi \cdot 1/4$ toluene (810.9): C 51.47, H 5.35, N 12.09; found: C 51.31, H 5.43, N 12.14.

6. *3′-O-[(*tert*-Butyl)dimethylsilyl]-*N^6*,2′-O-bis[(*tert*-butyloxy)carbonyl]adenosine* (**10**). A soln. of **7** (200 mg, 0.258 mmol) in 0.5M DBU/MeCN (5.2 ml) was stirred at r.t. for 18 h. The mixture was neutralized with 1M AcOH/MeCN (2.6 ml) and evaporated. Purification was achieved by FC (21 × 1.5 cm, toluene/AcOEt 3:1 (100 ml) and 2:1 (300 ml)): 116 mg (77%) of **10**. UV (MeOH): 271 (sh, 4.21), 266 (4.28), 210 (4.38). ^{1}H-NMR ($CDCl_3$): 8.72 (*s*, H–C(8)); 7.98 (*s*, H–C(2)); 7.96 (*s*, NH); 6.15 (*m*, OH–C(5′)); 6.07 (*d*, H–C(1′)); 5.60 (*m*, H–C(2′)); 4.73 (*d*, H–C(3′)); 4.22 (*s*, H–C(4′)); 3.96, 3.72 (2*m*, 2 H–C(5′)); 1.54 (*s*, *t*-Bu); 1.38 (*s*, *t*-Bu); 0.92 (*s*, *t*-BuSi); 0.10 (*s*, Me_2Si). Anal. calc. for $C_{26}H_{43}N_5O_8Si$ (581.7): C 53.68, H 7.45, N 12.04; found: C 54.03, H 7.62, N 11.66.

7. *3′-O-[(*tert*-Butyl)dimethylsilyl]-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**11**). 7.1. A soln. of **7** (100 mg, 0.129 mmol) in CF_3COOH (2 ml) was stirred at r.t. for 15 min. Then, the mixture was diluted with CH_2Cl_2 (60 ml) and washed with 3% $NaHCO_3$ soln. (2 × 30 ml), dried (Na_2SO_4), and evaporated. The crude product was purified by FC (8 × 1.5 cm, AcOEt/MeOH 95:5): 54 mg (73%) of **11**. UV (MeOH): 300 (sh, 3.51), 272 (sh, 4.40), 267 (4.44). ^{1}H-NMR ((D_6)DMSO): 10.62 (*s*, NH); 8.68 (*s*, H–C(8)); 8.62 (*s*, H–C(2)); 8.16 (*d*, 2 H *o* to NO_2); 7.61 (*d*, 2 H *m* to NO_2); 5.97 (*d*, H–C(1′)); 5.40 (br. *s*, OH–C(2′)); 5.20 (br. *s*, OH–C(5′)); 4.75 (*t*, H–C(2′)); 4.39 (*t*, OCH_2CH_2); 4.31 (*m*, H–C(3′)); 3.96 (*s*, H–C(4′)); 3.65, 3.56 (2*m*, 2 H–C(5′)); 3.11 (*t*, OCH_2CH_2); 0.90 (*s*, *t*-BuSi); 0.11 (*s*, Me_2Si). Anal. calc. for $C_{25}H_{34}N_6O_8Si \cdot H_2O$ (592.7): C 50.66, H 6.12, N 14.18; found: C 50.80, H 6.07, N 14.28.

7.2. At r.t., **4** (250 mg, 0.295 mmol) was stirred with 2% TsOH in CH_2Cl_2/MeOH 4:1 (5.9 ml) for 30 min. The mixture was then diluted with CH_2Cl_2 (40 ml), washed with $NaHCO_3$ soln. (40 ml) and H_2O (40 ml), dried (Na_2SO_4), and evaporated. The residue was purified by FC (15 × 1.5 cm, CH_2Cl_2/MeOH 98:2) and the product treated with little CH_2Cl_2/Et_2O 1:1.5 (*v:v*): 137 mg (81%) of **11**. Colourless powder.

8. *2′-O-[(*tert*-Butyl)dimethylsilyl]-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**12**). A soln. (6.5 ml) of 2% TsOH in CH_2Cl_2/MeOH 4:1 was stirred together with 273 mg (0.322 mmol) of **3**. After 20 min at r.t., the mixture was diluted with AcOEt (100 ml), the soln. washed twice with H_2O (60 ml), dried (Na_2SO_4), and evaporated, and the residue applied to FC (16 × 1.5 cm, toluene/AcOEt 1:1 (200 ml) and AcOEt (150 ml)): 168 mg (91%) of **12**. Colourless foam. UV (MeOH): 300 (sh, 3.49), 271 (sh, 4.40), 267 (4.43). ^{1}H-NMR ((D_6)DMSO): 10.61 (*s*, NH); 8.66 (*s*, H–C(8)); 8.58 (*s*, H–C(2)); 8.12 (*d*, 2 H *o* to NO_2); 7.57 (*d*, 2 H *m* to NO_2); 5.99 (*d*, H–C(1′)); 5.21 (*t*, OH–C(3′)); 5.17 (*d*, OH–C(5′)); 4.66 (*t*, H–C(2′)); 4.37 (*t*, OCH_2CH_2); 4.12 (*m*, H–C(3′)); 4.00 (*m*,

H–C(4′)); 3.72, 3.60 (2*m*, 2 H–C(5′)); 3.08 (*t*, OCH_2CH_2); 0.66 (*s*, *t*-BuSi); –0.14 (*s*, MeSi); –0.26 (*s*, MeSi). Anal. calc. for $C_{25}H_{34}N_6O_8Si$ (574.7): C 52.25, H 5.96, N 14.62; found: C 52.42, H 6.14, N 14.12.

9. *2′-O-[(*tert*-Butyl)dimethylsilyl]-5′-O-(monomethoxytrityl)adenosine* (**13**), *and 3′-O-[(*tert*-Butyl)dimethylsilyl]-5′-O-(monomethoxytrityl)adenosine* (**14**). A soln. of **4** (500 mg, 0.59 mmol) in 5 mM DBU/MeCN (24 ml) was stirred for 20 h, then neutralized by addition of 0.1M AcOH (12 ml), stirred for further 15 min, and evaporated. The residue was purified by FC (12 × 2.5 cm, CH_2Cl_2, CH_2Cl_2/MeOH 99:1 and 98:2): 158 mg (41%) of **13** and 170 mg (44%) of **14**. Overall yield 85%.

13: UV (MeOH): 259 (4.20), 233 (4.24). ^{1}H-NMR ($CDCl_3$): 8.27 (*s*, H–C(8)); 8.02 (*s*, H–C(2)); 7.48–7.23 (*m*, 12 H of MeO*Tr*); 6.82 (*d*, 2 H *o* to MeO); 6.02 (*d*, H–C(1′)); 5.64 (br. *s*, NH_2); 5.00 (*t*, H–C(2′)); 4.34 (*q*, H–C(3′)); 4.26 (*q*, H–C(4′)); 3.79 (*s*, MeO); 3.56–3.37 (*m*, 2 H–C(5′)); 2.74 (*d*, OH–C(3′)); 0.84 (*s*, *t*-BuSi); – 0.01 (*s*, MeSi); – 0.13 (*s*, MeSi). Anal. calc. for $C_{36}H_{43}N_5O_5Si$ (653.9): C 66.13, H 6.63, N 10.71; found: C 65.65, H 6.63, N 10.52.

14: UV (MeOH): 259 (4.17), 233 (4.21). ^{1}H-NMR ($CDCl_3$): 8.30 (*s*, H–C(8)); 8.06 (*s*, H–C(2)); 7.41–7.17 (*m*, 12 H of MeO*Tr*); 6.79 (*d*, 2 H *o* to MeO); 6.01 (*d*, H–C(1′)); 6.00 (*s*, NH_2); 4.72 (*t*, H–C(2′)); 4.60 (*q*, H–C(3′)); 4.18 (*q*, H–C(4′)); 3.77 (*s*, MeO); 3.58–3.23 (*m*, OH–C(2′)); 2 H–C(5′)); 0.87 (*s*, *t*-BuSi); 0.08 (*s*, MeSi); 0.00 (*s*, MeSi). Anal. calc. for $C_{36}H_{43}N_5O_5Si \cdot 1/2\ H_2O$ (671.9): C 65.23, H 6.69, N 10.57; found: C 65.54, H 6.90, N 10.48.

10. *5′-O-(Dimethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**18**). See [22]. After co-evaporation with dry pyridine (4 × 40 ml), **1** [13] [18] (3.62 g, 7.86 mmol) in dry pyridine (40 ml) and $(MeO)_2TrCl$ (3.2 g, 9.4 mmol) were stirred for 17 h at r.t. The mixture was diluted with MeOH (6 ml), evaporated, and co-evaporated with toluene (2 × 25 ml). Purification by CC (silica gel (40 × 4 cm), $CHCl_3$ (1 l), $CHCl_3$/MeOH 100:3 (1.8 l)) gave, after drying at 40°/high vacuum, 5.51 g (92%) of amorphous solid. UV (MeOH): 274 (sh, 4.39), 267 (4.44), 236 (4.40). ^{1}H-NMR ($CDCl_3$): 8.72 (*s*, H–C(8)); 8.20 (*s*, H–C(2)); 8.17 (*d*, 2 H *o* to NO_2); 8.02 (br. *s*, NH); 7.42 (*d*, 2 H *m* to NO_2); 7.21–7.11 (*m*, 9 H of $(MeO)_2Tr$); 6.71 (*d*, 4 H *o* to MeO); 5.98 (*d*, H–C(1′)); 5.66 (br. *s*, OH–C(3′)); 4.85 (*t*, H–C(2′)); 4.53 (*t*, OCH_2CH_2); 4.41 (*m*, H–C(3′), H–C(4′)); 3.74 (*s*, 2 MeO); 3.45–3.20 (*m*, 2 H–C(5′)); 3.14 (*t*, OCH_2CH_2); 3.06 (br. *s*, OH–C(2′)). Anal. calc. for $C_{40}H_{38}N_6O_{10} \cdot H_2O$ (780.79): C 61.53, H 5.16, N 10.76; found: C 61.29, H 5.34, N 10.74.

11. *2′-O-[(*tert*-Butyl)dimethylsilyl]-5′-O-(dimethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**19**), *3′-O-[(*tert*-Butyl)dimethylsilyl]-5′-O-(dimethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**20**), *and 2′,3′-Bis-O-[(*tert*-butyl)dimethylsilyl]-5′-O-(dimethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**21**). See [22]. After co-evaporation with abs. pyridine (4 × 20 ml), **18** (2.26 g, 2.96 mmol) was dissolved in abs. pyridine (18 ml). At r.t., (*t*-Bu)Me_2SiCl (532 mg, 3.55 mmol) and 1*H*-imidazole (483 mg, 7.1 mmol) were added, and the mixture was stirred for 26 h. Then, MeOH (6 ml) was added, the soln. evaporated to ¼ volume, diluted with CH_2Cl_2 (200 ml), washed twice with H_2O (100 ml), dried (Na_2SO_4), evaporated, and co-evaporated with toluene (4 × 10 ml) to remove pyridine. Purification by CC (silica gel (25.5 × 3 cm), toluene/AcOEt 10:1 (100 ml), 5:1 (200 ml), 4:1 (1.9 l), 10:3 (200 ml), 7:4 (600 ml), and 1:1 (600 ml)) gave, after drying under high vacuum, 244 mg (8%) of **21**, 857 mg (33%) of **19**, and 1.48 g (50%) of **20**. Overall yield 91%.

19: UV (MeOH): 274 (sh, 4.42), 267 (4.47), 236 (4.42). ^{1}H-NMR ($CDCl_3$): 8.65 (*s*, H–C(8)); 8.17 (*d*, 2 H *o* to NO_2); 8.16 (*s*, H–C(2)); 8.01 (*s*, NH); 7.44–7.22 (*m*, 9 H of $(MeO)_2Tr$, 2 H *m* to NO_2); 6.79 (*d*, 4 H *o* to MeO); 6.06 (*d*, H–C(1′)); 4.97 (*t*, H–C(2′)), 4.52 (*t*, OCH_2CH_2); 4.33 (*m*, H–C(3′)); 4.25 (*m*, H–C(4′)); 3.76 (*s*, 2 MeO); 3.55–3.34 (2 *m*, 2 H–C(5′)); 3.14 (*t*, OCH_2CH_2); 2.68 (*d*, OH–C(3′)); 0.81 (*s*, *t*-BuSi); – 0.04 (*s*, MeSi); – 0.19 (*s*, MeSi). Anal. calc. for $C_{46}H_{52}N_6O_{10}Si$ (877.0): C 63.00, H 5.98, N 9.58; found: C 62.80, H 6.01, N 9.31

20: UV (MeOH): 274 (sh, 4.41), 267 (4.46), 236 (4.42). ^{1}H-NMR ($CDCl_3$): 8.69 (*s*, H–C(8)); 8.45 (br. *s*, NH); 8.19 (*s*, H–C(2)); 8.14 (*d*, 2 H *o* to NO_2); 7.40–7.14 (*m*, 9 H of $(MeO)_2Tr$, 2 H *m* to NO_2); 6.77 (*d*, 4 H *o* to MeO); 6.01 (*d*, H–C(1′)); 4.74 (*q*, H–C(3′)); 4.57 (*t*, H–C(2′)); 4.50 (*t*, OCH_2CH_2); 4.17 (*m*, H–C(4′)); 3.75, 3.74 (2 *s*, 2 MeO); 3.53–3.21 (*m*, 2 H–C(5′)); 3.18 (*d*, OH–C(2′)); 3.11 (*t*, OCH_2CH_2); 0.87 (*s*, *t*-BuSi); 0.07 (*s*, MeSi); 0.00 (*s*, MeSi). Anal. calc. for $C_{46}H_{52}N_6O_{10}Si$ (877.0): C 63.00, H 5.98, N 9.58; found: C 62.99, H 6.04, N 9.11.

21: UV (MeOH): 274 (sh, 4.41), 267 (4.46), 236 (4.42). ^{1}H-NMR ($CDCl_3$): 8.63 (*s*, H–C(8)); 8.17 (*s*, H–C(2)); 8.16 (*d*, 2 H *o* to NO_2); 7.92 (*s*, NH); 7.46–7.20 (*m*, 9 H of $(MeO)_2Tr$, 2 H *m* to NO_2); 6.80 (*d*, 4 H *o* to MeO); 5.99 (*d*, H–C(1′)); 4.85 (*t*, H–C(2′)); 4.52 (*t*, OCH_2CH_2); 4.23 (*m*, H–C(3′), H–C(4′)); 3.76 (*s*, 2 MeO); 3.59–3.27 (*m*, 2 H–C(5′)); 3.14 (*t*, OCH_2CH_2); 0.84 (*s*, *t*-BuSi); 0.73 (*s*, *t*-BuSi); 0.03 (*s*, MeSi); – 0.03 (*s*, MeSi); – 0.09 (*s*, MeSi); – 0.35 (*s*, MeSi). Anal. calc. for $C_{52}H_{66}N_6O_{10}Si_2$ (991.3): C 63.01, H 6.71, N 8.48; found: C 62.79, H 6.81, N 8.45.

12. *2′-O-[(*tert*-Butyl)dimethylsilyl]-5′-O-(dimethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]-3′-O-[2-(4-nitrophenyl)ethylsulfonyl]adenosine* (**23**). To a soln. of **19** (2.63 g, 3 mmol) in abs. pyridine (30 ml), which was

first co-evaporated twice with abs. pyridine (20 ml), 2-(4-nitrophenyl)ethylsulfonyl chloride [14] [15] (1.5 g, 6 mmol) were added and stirred at r.t. for 75 min. Then, the mixture was evaporated to a small volume (10 ml) and co-evaporated with toluene (3 × 20 ml) to remove pyridine. The residue was purified by FC (20 × 2 cm, packed and eluted with toluene/AcOEt 4:1 (400 ml), then 3:1 (400 ml), 2:1 (300 ml), and 1:1 (150 ml)). Evaporation of product fractions (1.25 l) and co-evaporation with CH_2Cl_2 gave 3.22 g (98%) of **23**. Amorphous solid. UV (MeOH): 272 (sh, 4.56), 267 (4.59), 237 (4.47). ^{1}H-NMR ($CDCl_3$): 8.66 (*s*, H–C(8)); 8.21–8.11 (*m*, NH, H–C(2), 4 H *o* to NO_2); 7.45–7.15 (*m*, 9 H of $(MeO)_2Tr$, 4 H *m* to NO_2); 6.79 (*d*, 2 H *m* to MeO); 5.96 (*d*, H–C(1′)); 5.31 (*m*, H–C(2′)); 5.19 (*t*, H–C(3′)); 4.54 (*t*, OCH_2CH_2); 4.43 (*m*, H–C(4′)); 3.77 (2 *s*, 2 MeO); 3.68 (*m*, 1 H–C(5′)); 3.65–3.23 (*m*, 1 H–C(5′), $SO_2CH_2CH_2$); 3.16 (*t*, OCH_2CH_2); 0.73 (*s*, *t*-BuSi); – 0.01 (*s*, MeSi); – 0.28 (*s*, MeSi). Anal. calc. for $C_{54}H_{59}N_7O_{14}SSi$ (1090.2): C 59.49, H 5.45, N 8.99; found: C 59.94, H 5.56, N 8.74.

13. *5′-O-(Dimethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]-3′-O-[2-(4-nitrophenyl)ethylsulfonyl]-adenosine* (**24**). A soln. of **23** (2.98 g, 2.73 mmol) in THF (13.8 ml) was cooled in an ice/NaCl bath to – 10°, and then the soln. of $Bu_4NF \cdot 3\ H_2O$ (2.55 g, 8.1 mmol) in THF (5 ml) was added. The cooled mixture (– 5°) was stirred for 30 min, diluted with toluene/AcOEt 5:1 (5 ml), and applied immediately to FC (19 × 2.5 cm). Elution with CH_2Cl_2 (300 ml), then CH_2Cl_2/MeOH 99:1 (200 ml), 98.5:1.5 (400 ml; → **24**), 98:2 (400 ml; → **24**), and 97:3 (400 ml; → by-product **18**) gave, after evaporation and drying (high vacuum), 1.46 g (55%) of **24** and 585 mg (28%) of **18**. Overall yield 83%. **24**: UV (MeOH): 271 (sh, 4.56), 267 (4.58), 237 (4.47). ^{1}H-NMR ($CDCl_3$): 8.69 (*s*, H–C(8)); 8.21 (*s*, H–C(2)); 8.19 (*d*, 2 H *o* to NO_2); 8.18 (*d*, 2 H *o* to NO_2); 8.07 (br. *s*, NH); 7.44–7.09 (*m*, 4 H *m* to NO_2, 9 H of $(MeO)_2Tr$); 6.73 (*d*, 2 H *o* to MeO); 6.72 (*d*, 2 H *o* to MeO); 5.94 (*d*, H–C(1′)); 5.74 (*d*, OH–C(2′)); 5.24–5.15 (*m*, H–C(2′), H–C(3′)); 4.54 (*t*, OCH_2CH_2, H–C(4′)); 3.75 (2 *s*, 2 MeO); 3.64–3.27 (*m*, 2 H–C(5′), $SO_2CH_2CH_2$); 3.16 (*t*, OCH_2CH_2). Anal. calc. for $C_{48}H_{45}N_7O_{14}S \cdot H_2O$ (994.0): C 58.00, H 4.77, N 9.86; found: C 58.18, H 4.58, N 9.97.

14. *Bis(diisopropylamino)[2-(4-nitrophenyl)ethoxy]phosphane* (**26**). To a soln. of freshly dist. PCl_3 (28 ml, 280 mmol) in abs. Et_2O (80 ml) was added in small portions recrystallized 2-(4-nitrophenyl)ethanol [25] (8.35 g, 50 mmol) within 30 min at – 5° and under N_2. After stirring for 15 min at – 5° and 1.5 h at r.t., the solvent and excess of PCl_3 were evaporated under high vacuum. Then, the yellowish sirupy residue was dissolved in abs. Et_2O (200 ml), the soln. cooled to –10°, and $(i\text{-}Pr)_2NH$ (64 ml, 450 mmol) added dropwise within 30 min at –10° under N_2. The mixture was stirred for another 15 min at –10° and for 16 h at r.t. The voluminous precipitate of $(i\text{-}Pr)_2NH \cdot HCl$ was filtered under N_2 and the solvent evaporated. The yellowish sirupy product **26** (17.6 g, 89%), which crystallized on storage at – 20°; was pure enough to be used for phosphitylation reactions. ^{1}H-NMR ($CDCl_3$): 8.13–8.10 (*d*, 2 H *o* to NO_2); 7.40–7.36 (*d*, 2 H *m* to NO_2); 3.82–3.75 (*q*, $POCH_2CH_2$); 3.51–3.36 (*m*, 2 Me_2CH); 3.00–2.95 (*t*, $POCH_2CH_2$); 1.12–1.05 (2 *d*, 2 Me_2CH). ^{31}P-NMR ($CDCl_3$): 123.53.

15. *3′-O-[(tert-Butyl)dimethylsilyl]-5′-O-(monomethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 2′-[2-(4-Nitrophenyl)ethyl N,N-Diisopropylphosphoramidite]* (**27**). To a soln. (5 ml) of **4** (847 mg, 1 mmol) in abs. MeCN, **26** (795 mg, 2 mmol) and 1*H*-tetrazole (35 mg, 0.5 mmol) were added under N_2. The mixture was stirred for 16 h at r.t., then diluted with CH_2Cl_2 (2 × 50 ml), and washed with sat. $NaHCO_3$/NaCl soln. (2 × 50 ml). The org. phase was dried (Na_2SO_4), evaporated, and purified by FC (10 × 2.5 cm, toluene/AcOEt 1:1 (250 ml)). The product fraction (100 ml) was evaporated, co-evaporated with CH_2Cl_2, and dried (30°/high vacuum): 1.06 g (92%) of **27**. Amorphous solid. UV (MeOH): 272 (sh, 4.53), 268 (4.56), 234 (sh, 4.35). ^{31}P-NMR ($CDCl_3$): 150.33, 150.21. ^{1}H-NMR ($CDCl_3$): 8.67, 8.65, (2*s*, H–C(8), diast.); 8.18 (*d*, 2 H *o* to NO_2); 8.13–7.96 (*m*, H–C(2), 2 H *o* to NO_2); 7.89 (br. *s*, NH); 7.46–7.15 (*m*, 4 H *m* to NO_2), 12 H of MeO*Tr*); 6.79, 6.77 (2*d*, 2 H *o* to MeO, diast.); 6.14 (*m*, H–C(1′)); 5.05, 4.88 (2*m*, H–C(2′), diast.); 4.53 (*m*, H–C(3′), OCH_2CH_2); 4.23 (*m*, H–C(4′)); 3.78, 3.77 (2*s*, MeO, diast.); 3.89–3.13 (*m*, $POCH_2CH_2$, 2 Me_2CH, 2 H–C(5′)); 3.16 (*t*, OCH_2CH_2, diast.); 2.92, 2.72 (2*t*, $POCH_2CH_2$, diast.); 1.32, 1.00 (2*m*, 2 Me_2CH); 0.84 (*s*, *t*-BuSi); 0.06 (*s*, MeSi); 0.00 (*s*, MeSi). Anal. calc. for $C_{59}H_{71}N_8O_{12}PSi$ (1143.3): C 61.9, H 6.26, N 9.80; found: C 61.86, H 6.41, N 9.48.

16. *3′-Deoxy-5′-O-(monomethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 2′-[2-(4-Nitrophenyl)ethyl N,N-Diisopropylphosphoramidite]* (**28**). To a soln. of **17** [21] (1.44 g, 2 mmol) and 1*H*-tetrazole (70 mg, 1 mmol) in abs. MeCN (10 ml), **26** (1.59 g, 4 mmol) was added under N_2. After stirring for 80 min at r.t., the mixture was diluted with CH_2Cl_2 (200 ml), the soln. washed with sat. $NaHCO_3$/NaCl soln. (2 × 100 ml), dried (Na_2SO_4), and evaporated, and the residue purified by FC (19 × 2.5 cm, packed and eluted with toluene/AcOEt 1:1 (400 ml)). The product fraction (200 ml) was evaporated, co-evaporated with CH_2Cl_2 (3 × 20 ml), and dried at 30°/high vacuum: 1.8 g (89%) of **28**. UV (MeOH): 268 (4.57), 233 (sh, 4.35). ^{1}H-NMR ($CDCl_3$): 8.69, 8.68 (2*s*, H–C(8), diast.); 8.20–8.00 (*m*, H–C(2), NH, 4 H *o* to NO_2); 7.47–7.16 (*m*, 4 H *m* to NO_2, 12 H of MeO*Tr*); 6.82, 6.81 (2*d*, 2 H *o* to MeO, diast); 6.15 (*s*, H–C(1′)); 4.90 (*m*, H–C(2′)); 4.63 (*m*, H–C(4′)); 4.53 (*t*, OCH_2CH_2); 3.79,

3.78 (2*s*, MeO, diast.); 3.92–3.31 (*m*, $POCH_2CH_2$, 2 Me_2CH, 2 H–C(5′)); 3.16 (2*t*, OCH_2CH_2, diast.); 2.98 (2*m*, $POCH_2CH_2$, diast.); 2.29, 2.10 (2*m*, 2 H–C(3′)); 1.12 (*s*, Me_2CH); 1.09 (*s*, Me_2CH). ^{31}P-NMR ($CDCl_3$): 149.78, 148.85. Anal. calc. for $C_{53}H_{57}N_8O_{11}P$ (1013.1): C 62.84, H 5.67, N 11.06; found: C 62.60, H 5.80, N 10.83.

17. *5′-O-(Monomethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]-3′-O-[2-(4-nitrophenyl)ethylsulfonyl]adenosine 2′-[2-Cyanoethyl* N,N-*Diisopropylphosphoramidite]* (**29**). To **22** (770 mg, 0.81 mmol) in abs. CH_2Cl_2 (10 ml) and abs. MeCN (1 ml), 600 mg (2.54 mmol) of chloro(2-cyanoethoxy)(diisopropylamino)phosphane [24] (**25**), and 0.57 ml of $(i\text{-Pr})_2EtN$ (*Hünig*'s base) were added under N_2. After stirring for 2.25 h at r.t., the mixture was diluted with AcOEt, and washed with sat. $NaHCO_3$/NaCl soln., dried (Na_2SO_4), and evaporated. Purification was achieved by FC (19 × 2.5 cm, AcOEt (400 ml)). The product fraction (200 ml) was evaporated and dried at r.t./high vacuum: 842 mg (90%) of amorphous solid. UV (MeOH): 272 (sh, 4.49), 267 (4.52), 234 (4.32). ^{1}H-NMR ($CDCl_3$): 8.65, 8.62 (2*s*, H–C(8), diast.); 8.18–8.07 (*m*, H–C(2), 4 H *o* to NO_2); 7.96 (br. *s*, NH); 7.43–7.14 (*m*, 4 H *m* to NO_2, 12 H of MeO*Tr*); 6.77, 6.74 (2*d*, 4 H *o* to MeO); 6.13, 6.08 (2*d*, H–C(1′), diast.); 5.60–5.23 (3*m*, H–C(2′), H–C(3′), diast.); 4.52 (*t*, OCH_2CH_2); 4.46, 4.39 (2*m*, H–C(4′), diast.); 3.76, 3.75 (2*s*, 2 MeO); 3.88–3.21 (*m*, 2 Me_2CH, $POCH_2CH_2$, 2 H–C(5′), $SO_2CH_2CH_2$); 3.14 (*t*, OCH_2CH_2); 2.62–2.33 (*m*, $POCH_2CH_2$, diast.); 1.30–0.99 (*m*, Me_2CH, *Me*CH); 0.72 (*d*, *Me*CH). ^{31}P-NMR ($CDCl_3$): 153.87, 151.91. Anal. calc. for $C_{56}H_{60}N_9O_{14}PS$ (1146.2): C 58.68, H 5.28, N 11.00; found: C 57.54, H 5.85, N 11.12.

18. *5′-O-(Dimethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]-3′-O-[2-(4-nitrophenyl)ethylsulfonyl]-adenosine 2′-[2-Cyanoethyl* N,N-*Diisopropylphosphoramidite]* (**30**). The suspension of **24** (976 mg, 1 mmol) in abs. MeCN (5 ml) was dissolved by addition of abs. CH_2Cl_2 (5 ml). Then, **25** (710 mg, 3 mmol) and $(i\text{-Pr})_2EtN$ were added under N_2. The mixture was stirred at r.t. for 3 h, diluted with CH_2Cl_2 (150 ml), washed with sat. $NaHCO_3$/NaCl soln. (60 ml), dried (Na_2SO_4), and evaporated. Purification by FC (16 × 2.5 cm, toluene/AcOEt 1:1 (500 ml)) gave 1.02 g (86%) of colourless solid foam. UV (MeOH): 271 (sh, 4.56), 267 (4.59), 237 (4.47). ^{1}H-NMR ($CDCl_3$): 8.68, 8.65 (2*s*, H–C(8), diast.); 8.20–8.09 (*m*, H–C(2), 4 H *o* to NO_2); 8.07 (br. *s*, NH); 7.45–7.16 (*m*, 4 H *m* to NO_2, 12 H of $(MeO)_2Tr$); 6.76 (*m*, 4 H *o* to MeO); 6.14, 6.09 (2*d*, H–C(1′), diast.); 5.60–5.23 (3*m*, H–C(2′), H–C(3′), diast.); 4.54 (*t*, OCH_2CH_2); 4.47, 4.40 (2*m*, H–C(4′), diast.); 3.77, 3.76 (2*s*, 2 MeO); 3.89–3.22 (*m*, 2 Me_2CH, $POCH_2CH_2$, 2 H–C(5′), $SO_2CH_2CH_2$); 3.15 (*t*, OCH_2CH_2); 2.52, 2.34 (*t* + *m*, $POCH_2CH_2$, diast.); 1.31–0.98 (*m*, Me_2CH, *Me*CH); 0.73 (*d*, *Me*CH). ^{31}P-NMR ($CDCl_3$): 154.07, 152.14. Anal. calc. for $C_{57}H_{62}N_9O_{15}PSi$ (1176.2): C 58.21, H 5.31, N 10.72; found: C 57.81, H 5.51, N 10.45.

19. *5′-O-(Dimethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]-3′-O-[2-(4-nitrophenyl)ethylsulfonyl]-adenylyl-{2′-[O^P-(2-cyanoethyl)]-5′}-3′-O-[(*tert*-butyl)dimethylsilyl]-N^6,2′-O-bis[(*tert*-butyloxy)carbonyl]-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**31**). To a soln. of **7** (344 mg, 0.444 mmol) in abs. MeCN (4.3 ml), **30** (752 mg, 0.639 mmol) and 1*H*-tetrazole (90 mg, 1.28 mmol) were added under N_2. At r.t., the mixture was stirred for 4.5 h, oxidized with I_2/H_2O/pyridine (I_2 (0.5 mg) in pyridine/H_2O/CH_2Cl_2 3:1:1 (5 ml)), then stirred for another 15 min, diluted with CH_2Cl_2 (40 ml), and washed twice with sat. $Na_2S_2O_3$/NaCl soln. (40 ml). The aq. phase was reextracted with CH_2Cl_2 (2 × 30 ml), the combined CH_2Cl_2 phase evaporated and co-evaporated with toluene (3 × 30 ml), and the residue purified by FC (14 × 2.5 cm) with toluene/AcOEt 4:1 (200 ml), 3:1 (200 ml; → **7**), 2:1 (200 ml), 1:2 (300 ml), 1:3 (200 ml; → **31**), 1:5 (400 ml; → **3**). The product fractions were evaporated and dried (r.t./high vacuum): 510 mg (62%, 78% based on recovered **7**) of **31** and 74 mg (22%) of **7**. UV (MeOH): 267 (4.74), 239 (sh, 4.55). ^{1}H-NMR ($CDCl_3$): 8.82 (*m*, H–C(8), diast.); 8.67 (*d*, H–C(8), diast.); 8.25–7.98 (*m*, 2 H–C(2), 2 NH, 6 H *o* to NO_2); 7.45 (*d*, 2 H *m* to NO_2); 7.30–7.10 (*m*, 4 H *m* to NO_2, 9 H of $(MeO)_2Tr$); 6.72 (*m*, 4 H *o* to MeO); 6.19 (*m*, 2 H–C(1′)); 5.98–5.71 (*m*, 2 H–C(2′), diast.); 5.63 (*t*, H–C(3′)); 4.77 (*q*, H–C(3′)); 4.54 (*t*, OCH_2CH_2); 4.45–3.19 (*m*, OCH_2CH_2, 2 H–C(4′), $POCH_2CH_2$, 4 H–C(5′), $SO_2CH_2CH_2$); 3.74 (2*s*, 2 MeO); 3.17 (*t*, OCH_2CH_2); 2.97 (*q*, OCH_2CH_2); 2.65, 2.50 (2*t*, $POCH_2CH_2$); 1.41 (2*s*, 2 *t*-Bu); 0.92 (*s*, *t*-BuSi); 0.11 (*s*, MeSi); 0.10 (*s*, MeSi). ^{31}P-NMR ($CDCl_3$): –1.73, –1.78. Anal. calc. for $C_{86}H_{97}N_{14}O_{28}PSSi$ (1865.9): C 55.36, H 5.24, N 10.51; found: C 55.33, H 5.38, N 10.53.

20. *N^6-[2-(4-Nitrophenyl)ethoxycarbonyl]-3′-O-[2-(4-nitrophenyl)ethylsulfonyl]adenylyl-{2′-[O^P-(2-cyanoethyl)]-5′}-3′-O-[(*tert*-butyl)dimethylsilyl]-N^6,2′-O-bis[(*tert*-butyloxy)carbonyl]-N^6-[2-(4-nitrophenyl)-ethoxycarbonyl]adenosine* (**32**). A soln. (12 ml) of 3% CCl_3COOH in CH_2Cl_2 was stirred together with 438 mg (0.235 mmol) of **31** at 5°. After 8 min, detritylation was stopped by addition of sat. $NaHCO_3$ soln. (60 ml). The mixture was washed with CH_2Cl_2 (3 × 60 ml), dried (Na_2SO_4), and evaporated. FC (12 × 1.5 cm) with CH_2Cl_2 (50 ml), CH_2Cl_2/MeOH 99:1 (100 ml), 98:2 (200 ml), and 97:3 (100 ml) gave 314 mg (86%) of **32** as foam, after evaporation and drying (r.t./high vacuum). UV (MeOH): 267 (4.77). ^{1}H-NMR ($CDCl_3$): 8.81 (*s*, H–C(8)); 8.69 (*d*, H–C(8)); 8.30 (*d*, H–C(2)); 8.24–8.16 (*m*, 6 H *o* to NO_2); 8.06–8.01 (*m*, H–C(2), 2 NH); 7.44 (*m*, 4 H *o* to NO_2);

7.20, 7.17 (2*d*, 2 H *o* to NO_2); 6.19 (*m*, H–C(1′)); 6.03 (*m*, H–C(1′)); 5.72–5.56 (*m*, 2 H–C(2′), OH–C(5′)); 5.44, 5.28 (2*m*, H–C(3′), diast.); 4.79 (*m*, H–C(3′)); 4.54 (*t*, OCH_2CH_2); 4.46 (*m*, OCH_2CH_2, H–C(4′)); 4.24–3.26 (*m*, H–C(4′), 4 H–C(5′), $POCH_2CH_2$, $SO_2CH_2CH_2$, OCH_2CH_2); 3.17 (*dt*, OCH_2CH_2); 3.01, 2.54 (2*m*, $POCH_2CH_2$); 1.43 (*s*, *t*-Bu); 1.42 (*s*, *t*-Bu); 0.91 (*s*, *t*-BuSi); 0.11 (*s*, MeSi); 0.09 (*s*, MeSi). Anal. calc. for $C_{65}H_{79}N_{14}O_{26}PSSi$ (1563.5): C 49.93, H 5.09, N 12.54; found: C 49.78, H 5.12, N 12.62.

21. *5′-O-(Monomethoxytrityl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-3′-O-[2-(4-nitrophenyl)ethylsulfonyl]-adenylyl-{2′-[*O^P*-(2-cyanoethyl)]-5′}-3′-O-[(*tert*-butyl)dimethylsilyl]-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-2′-O-[2-(4-nitrophenyl)ethylsulfonyl]adenosine* (**33**). A mixture of **29** (767 mg, 0.668 mmol) and **9** (375 mg, 0.48 mmol) was dissolved in abs. MeCN (4.7 ml) under N_2 in presence of 1*H*-tetrazole (94 mg, 1.33 mmol). The soln. was stirred at r.t. for 4.5 h. Then, the intermediate was oxidized with I_2/H_2O/pyridine (see *Exper. 18*) until the brown colour persisted. After stirring at r.t. for another 10 min, the mixture was diluted with AcOEt (100 ml), washed twice with sat. $Na_2S_2O_3$/NaCl soln. (40 ml), dried (Na_2SO_4), evaporated, and co-evaporated with toluene (3 × 30 ml) to remove pyridine. The residue was dissolved in little CH_2Cl_2 and applied to FC (15 × 2.5 cm, packed and eluted with toluene/AcOEt 4:1 (300 ml), then 3:1 (400 ml), 1:1 (400 ml), 1:2 (300 ml; → **9**), 1:3 (400 ml), 1:4 (200 ml), 1:6 (200 ml; → **33**), and finally with AcOEt (500 ml)). Separate evaporation, and co-evaporation with CH_2Cl_2 gave 487 mg (55%; 84% based on recovered **9**) of **33** and 128 mg (34%) of **9**. UV (MeOH): 298 (sh, 3.74), 272 (sh, 4.44), 267 (4.48), 238 (sh, 4.16). ^{1}H-NMR ($CDCl_3$): 8.83–8.62 (*m*, 2 H–C(8)); 8.26–8.05 (*m*, 2 H–C(2), 2 NH, 8 H *o* to NO_2); 7.46–7.15 (*m*, 12 H of MeO*Tr*, 8 H *m* to NO_2); 6.73 (*d*, 2 H *o* to MeO); 6.26–6.13 (*m*, 2 H–C(1′)); 5.95–5.53 (*m*, 2 H–C(2′), H–C(3′)); 4.75–4.70 (*m*, H–C(3′)); 4.54 (*m*, 2 OCH_2CH_2); 3.75 (*s*, MeO); 4.48–3.22 (*m*, 2 H–C(4′), 4 H–C(5′), 2 $SO_2CH_2CH_2$); 3.16 (*m*, 2 OCH_2CH_2); 2.75–2.54 (*m*, $POCH_2CH_2$); 0.95, 0.92 (2*s*, *t*-BuSi, diast.); 0.19–0.11 (*m*, Me_2Si). Anal. calc. for $C_{83}H_{86}N_{15}O_{27}PS_2Si$ (1848.9): C 53.92, H 4.69, N 11.36; found: C 53.48, H 4.99, N 11.40.

22. N^6*-[2-(4-Nitrophenyl)ethoxycarbonyl]-3′-O-[2-(4-nitrophenyl)ethylsulfonyl]adenylyl-{2′-[*O^P*-(2-cyanoethyl)]-5′}-3′-O-[(*tert*-butyl)dimethylsilyl]-*N^6*-(2-(4-nitrophenyl)ethoxycarbonyl]-2′-O-[2-(4-nitrophenyl)ethylsulfonyl]adenosine* (**34**). A soln. of **33** (420 mg, 0.227 mmol) in 12 ml of 3% CCl_3COOH in CH_2Cl_2 was stirred for 45 min on ice-cooling and at r.t. for another 30 min. Then, the mixture was diluted with AcOEt (150 ml), the soln. washed with sat. $NaHCO_3$ soln. (2 × 80 ml), dried (Na_2SO_4), and evaporated, and the residue purified by FC (21 × 1.5 cm) with toluene/AcOEt 1:1 and 1:2 (each 100 ml), AcOEt (200 ml), and AcOEt/MeOH 9:1 (200 ml) and 7:3 (100 ml). The product fraction was evaporated and dried at 40°/high vacuum: 316 mg (88%) of **34**. Solid foam. UV (MeOH): 294 (sh, 4.04), 272 (sh, 4.62), 267 (4.66), 209 (sh, 4.69). ^{1}H-NMR ($CDCl_3$): 8.69–8.06 (*m*, 2 H–C(8), 2 H–C(2), 2 NH, 8 H *o* to NO_2); 7.46–7.28 (*m*, 8 H *m* to NO_2); 6.27–5.38 (*m*, 2 H–C(1′), 2 H–C(2′), H–C(3′), diast.); 4.78–2.54 (*m*, H–C(3′), 2 OCH_2CH_2, 2 H–C(4′), $POCH_2CH_2$, 4 H–C(5′), OH–C(5′), 2 OCH_2CH_2, $POCH_2CH_2$, 2 $SO_2CH_2CH_2$); 0.95–0.91 (2*s*, *t*-BuSi, diast.); 0.18–0.10 (*m*, Me_2Si, diast.). Anal. calc. for $C_{63}H_{74}N_{15}O_{26}PS_2Si$ (1580.5): C 47.88, H 4.72, N 13.29; found: C 48.03, H 4.82, N 13.17.

23. *3′-O-[(*tert*-Butyl)dimethylsilyl]-5′-O-(monomethoxytrityl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{*O^P*-[2-(4-nitrophenyl)ethyl]}-5′}-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-3′-O-[2-(4-nitrophenyl)ethylsulfonyl]adenylyl-{2′-[*O^P*-(2-cyanoethyl)]-5′}-3′-O-[(*tert*-butyl)dimethylsilyl]-*N^6*,2′-O-bis[(*tert*-butyloxy)carbonyl]-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**35**). To the soln. of **32** (100 mg, 0.064 mmol) in abs. MeCN (2 ml), **27** (146 mg, 0.128 mmol) and 1*H*-tetrazole (18 mg, 0.256 mmol) were added under N_2. After 3.5 h at r.t., 1*H*-tetrazole (9 mg, 0.128 mmol) and **27** (0.064 mmol) were added again, and the mixture was stirred for another 1.5 h. Following oxidation with I_2/H_2O/pyridine (see *Exper. 18*), the soln. was stirred for another 10 min. Then, the coloured soln. was extracted with CH_2Cl_2 (120 ml) and sat. $Na_2S_2O_3$/NaCl soln. (2 × 50 ml), dried (Na_2SO_4), evaporated, and co-evaporated with toluene (3 × 20 ml) to remove pyridine. The crude trimer **35** was purified by FC (15 × 1.5 cm, CH_2Cl_2/MeOH 99:1 (100 ml), 98:2 (300 ml), and 97:3 (200 ml)). The product was dried at 40°/high vacuum: 159 mg (95%) of amorphous solid. UV (MeOH): 267 (4.86), 236 (sh, 4.51). Anal. calc. for $C_{118}H_{135}N_{21}O_{39}P_2SSi_2$ (2621.7): C 54.06, H 5.19, N 11.22; found: C 54.22, H 5.30, N 11.15.

24. *3′-Deoxy-5′-O-(monomethoxytrityl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{*O^P*-[2-(4-nitrophenyl)ethyl]}-5′}-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-3′-O-[2-(4-nitrophenyl)ethylsulfonyl]adenylyl-{2′-[*O^P*-(2-cyanoethyl)]-5′}-3′-O-[(*tert*-butyl)dimethylsilyl]-*N^6*,2′-O-bis[(*tert*-butyloxy)carbonyl]-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**36**). As described in *Exper. 22*, with **32** (100 mg, 0.064 mmol), abs. MeCN (2 ml), **28** (195 mg, 0.192 mmol) and 1*H*-tetrazole (27 mg, 0.384 mmol; 5 h, r.t.). Oxidation, workup, and purification gave 95 mg (60%) of solid foam. UV (MeOH): 267 (4.95), 237 (sh, 4.62). Anal. calc. for $C_{112}H_{121}N_{21}O_{38}P_2SSi$ 2491.4): C 53.99, H 4.90, N 11.81; found: C 53.68, H 4.93, N 11.65.

25. *3′-O-[(*tert*-Butyl)dimethylsilyl]-5′-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]-3′-O-[2-(4-nitrophenyl)ethylsulfonyl]adenylyl-{2′-[O^P-(2-cyanoethyl)]-5′}-3′-O-[(*tert*-butyl)dimethylsilyl]-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]-2′-O-[2-(4-nitrophenyl)ethylsulfonyl]adenosine* (**37**). A soln. of **34** (100 mg, 0.064 mmol) in abs. MeCN (2 ml), **27** (219 mg, 0.192 mmol), and 1*H*-tetrazole (27 mg, 0.384 mmol) was stirred for 6 h at r.t. under N_2. After dropwise addition of I_2/H_2O/pyridine and stirring for another 10 min, the brown soln. was diluted with AcOEt (100 ml), washed with sat. $Na_2S_2O_3$/NaCl soln. (2 × 60 ml), dried (Na_2SO_4), evaporated, and co-evaporated with toluene (2 × 30 ml). The residue was purified by FC (12 × 1.5 cm, toluene/AcOEt 5:4, then toluene/AcOEt/MeOH 5:4:0.1, 5:4:0.5, and 5:4:1) and the product dried at 40°/high vacuum: 137 mg (82%) of **37**. Amorphous solid. UV (MeOH): 272 (sh, 4.94), 267 (4.98), 236 (sh, 4.60). Anal. calc. for $C_{116}H_{130}N_{22}O_{39}P_2S_2Si_2$ · toluene (2730.8): C 54.10, H 5.10, N 11.28; found: C 54.14, H 5.46, N 10.70.

26. *3′-Deoxy-5′-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]-3′-O-[2-(4-nitrophenyl)ethylsulfonyl]adenylyl-{2′-[O^P-(2-cyanoethyl)]-5′}-3′-O-[(*tert*-butyl)dimethylsilyl]-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]-2′-O-[2-(4-nitrophenyl)ethylsulfonyl]adenosine* (**38**). Analogously to *Exper. 24*, with **34** (100 mg, 0.064 mmol), abs. MeCN (2 ml), **28** (195 mg, 0.192 mmol), and 1*H*-tetrazole (27 mg, 0.384 mmol; 6 h, r.t.). Oxidation, workup, and purification gave 138 mg (87%) of solid foam. UV (MeOH): 298 (sh, 4.24), 272 (sh, 4.87), 267 (4.90), 236 (sh, 4.52). Anal. calc. for $C_{110}H_{116}N_{22}O_{38}P_2S_2Si$ (2508.4): C 52.67, H 4.66, N 12.28; found: C 52.97, H 4.76, N 11.58.

27. *3′-O-[(*tert*-Butyl)dimethylsilyl]-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]-3′-O-[2-(4-nitrophenyl)ethylsulfonyl]adenylyl-{2′-[O^P-(2-cyanoethyl)]-5′}-3′-O-[(*tert*-butyl)dimethylsilyl]-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]-2′-O-[2-(4-nitrophenyl)-ethylsulfonyl]adenosine* (**39**). A soln. of 2% TsOH in CH_2Cl_2/MeOH 4:1 (1.8 ml) and **37** (120 mg, 0.0455 mmol) were stirred for 1 h at r.t. Then the mixture was diluted with CH_2Cl_2 (100 ml), the soln. washed with phosphate buffer (0.15M, pH 6.8; 80 ml), dried (Na_2SO_4), and evaporated, and the residue purified by FC (10 × 1.5 cm) with CH_2Cl_2 (50 ml), then CH_2Cl_2/MeOH 99:1 (100 ml), 97:3 (200 ml), and 95:5 (100 ml). The product was dried under high vacuum at 30°: 100 mg (93%) of **39**. Solid foam. UV (MeOH): 296 (sh, 4.38), 272 (sh, 4.97), 267 (5.00), 210 (sh, 5.01). Anal. calc. for $C_{96}H_{110}N_{22}O_{38}P_2S_2Si_2$ (2362.3): C 48.81, H 4.69, N 13.04; found: C 48.97, H 4.90, N 12.68.

28. *3′-Deoxy-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]-3′-O-[2-(4-nitrophenyl)ethylsulfonyl]adenylyl-{2′-[O^P-(2-cyanoethyl)]-5′}-3′-O-[(*tert*-butyl)dimethylsilyl]-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]-2′-O-[2-(4-nitrophenyl)ethylsulfonyl]adenosine* (**40**). As described in *Exper. 26*, with **38** (119 mg, 0.047 mmol) and 2% TsOH in CH_2Cl_2/MeOH 4:1 (1.8 ml; 1 h, r.t.). Workup and purification by FC yielded 78 mg (74%) of **40**. Solid foam. UV (MeOH): 296 (sh, 4.39), 272 (sh, 4.98), 267 (5.01), 208 (sh, 5.02). Anal. calc. for $C_{90}H_{100}N_{22}O_{37}P_2S_2Si$ (2236.1): C 48.34, H 4.51, N 13.78; found: C 48.32, H 4.44, N 13.74.

29. *Isomer Mixture 3′-O-[(*tert*-Butyl)dimethylsilyl]adenylyl-(2′–5′)-adenylyl-(2′–5′)-3′-O-[(*tert*-butyl)-dimethylsilyl]adenosine Bis(triethylammonium) Salt* (**41**) *and 3′-O-[(*tert*-Butyl)dimethylsilyl]adenylyl-(2′–5′)-adenylyl-(2′–5′)-2′-O-[(*tert*-butyl)dimethylsilyl]adenosine Bis(triethylammonium) Salt* (**42**). At r.t., **39** (45 mg, 0.019 mmol) was stirred for 18 h with 0.5M DBU/MeCN (5.4 ml). Then the soln. was neutralized with 1M AcOH/MeCN (2.7 ml), diluted with CH_2Cl_2 (50 ml), and washed with H_2O (6 × 40 ml). The aq. phase was collected, evaporated to a small volume (20 ml), then applied onto a *DEAE-Sephadex* column *A 25* (60 × 1 cm), and eluted first with H_2O (500 ml), followed by a linear gradient of 0.0–0.2M $(Et_3NH)HCO_3$ buffer (pH 7.45) within 3000 ml. The eluate with 0.140–0.176M buffer was evaporated and co-evaporated with MeOH (8 × 20 ml). The residual Et_3NH^+ salts were lyophilised (H_2O): 12.6 mg (49%) of **41**/**42**. The isolated powder was insoluble in H_2O, but soluble in DMSO and MeCN/$(Et_3NH)OAc$ buffer. UV (buffer): 258. ^{1}H-NMR ((D_6)DMSO): 12.14 (br. *s*, 2 Et_3NH); 8.41–8.06 (*m*, 3 H–C(8), 3 H–C(2)); 7.72 (*m*, NH_2); 7.28–7.22 (br. *s*, 2 NH_2); 6.02–5.84 (*m*, 3 H–C(1′)); 0.88–0.70 (*s* + *m*, 2 *t*-BuSi); 0.11 (*s*, Me_2Si); 0.05 to –0.20 (3*s*, Me_2Si, isom.). HPLC (see *General*): t_R 13.74 and 15.04 min (isomer mixture **41**/**42**).

30. *Isomer Mixture 3′-Deoxyadenylyl-(2′–5′)-adenylyl-(2′–5′)-3′-O-[(*tert*-butyl)dimethylsilyl]adenosine Bis(triethylammonium) Salt* (**43**) *and 3′-Deoxyadenylyl-(2′–5′)-adenylyl-(2′–5′)-2′-O-[(*tert*-butyl)dimethylsilyl]-adenosine Bis(triethylammonium) Salt* (**44**). At r.t., the soln. of **40** (40 mg, 0.018 mmol) in 0.5M DBU/MeCN (5 ml) was stirred for 17 h, then neutralized with 1M AcOH in MeCN (2.5 ml), stirred for another 10 min, finally diluted with H_2O (90 ml), and applied onto a *DEAE-Sephadex* column *A 25* (60 × 1 cm) using first H_2O (500 ml) for desalting, followed by a linear gradient of $(Et_3NH)HCO_3$ buffer (pH 7.45; 0.0–0.2M) within 3000 ml. The eluate with 0.137–0.154M buffer was evaporated and co-evaporated 6 times with MeOH (20 ml). The residual Et_3NH^+

salts were lyophilised (H_2O): 12.9 mg (51%) of **43**/**44**. The isolated solid was insoluble in H_2O, but soluble in DMSO and buffers. UV (buffer): 257. ^{1}H-NMR (D_2O/(D_6)DMSO): 8.16–7.84 (*m*, 3 H–C(8), 3 H–C(2)); 5.99 (2*s*, H–C(1'), isom.); 5.90 (*m*, H–C(1'), isom.); 5.77, 5.73 (2*d*, H–C(1'), isom.); 2.40–2.17 (*m*, 2 H–C(3')); 0.86, 0.57 (2*s*, *t*-BuSi, isom.); 0.09 to – 0.33 (*d* + 2*s*, Me_2Si; isom.). HPLC (see *General*): 13.82 and 15.16 min (isomer mixture **43**/**44**).

31. *3'-O-[(*tert*-Butyl)dimethylsilyl]-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2'-{O^P-[2-(4-nitrophenyl)ethyl]}-5'}-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]-3'-O-[2-(4-nitrophenyl)ethylsulfonyl]adenylyl-{2'-[O^P-(2-cyanoethyl)]-5'}-3'-O-[(*tert*-butyl)dimethylsilyl]-N^6,2'-O-bis[(*tert*-butyloxy)carbonyl]-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**45**). At r.t., **35** (136 mg, 0.052 mmol) was detritylated by treatment with 2% TsOH in CH_2Cl_2/MeOH 4:1 (2.1 ml) for 45 min. Then, the mixture was diluted with CH_2Cl_2 (50 ml), the soln. washed twice with phosphate buffer (0.15M, pH 6.88), the aq. phase washed with CH_2Cl_2 (3 × 50 ml), the combined org. phase dried (Na_2SO_4) and evaporated, and the residue purified by FC (15 × 1.5 cm, packed and eluted with CH_2Cl_2/MeOH 99:1, then 98:2, 97:3, and 95:5 (each 100 ml)). Evaporation of the product fraction (100 ml) and co-evaporation with CH_2Cl_2 gave 97 mg (80%) of **45**. Amorphous solid. UV (MeOH): 300 (sh, 4.20), 272 (sh, 4.93), 267 (4.96). Anal. calc. for $C_{98}H_{119}N_{21}O_{38}P_2SSi_2$ (2349.3): C 50.10, H 5.11, N 12.52; found: C 49.94, H 5.22, N 12.70.

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