35. Nucleotides

Part XXXVIII[1])

Syntheses and Characterization of Phosphorothioate Analogues of (2′–5′)Adenylate Dimer and Trimer and Their 5′-*O*-Monophosphates

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Dedicated to Prof. Dr. *Wilhelm Fleischhacker* on the occasion of his 60th birthday

(8.XI.91)

The chemical syntheses of the phosphorothioate of (2′–5′)adenylate dimer (see **6a**, **6b**) and trimer (see **11a**, **11b**, **12a**, **12b**) as well as of their 5′-monophosphates (see **15a**, **15b**, **16a**, **16b**) using the phosphoramidite method are described. The resulting diastereoisomer mixtures were separated into the pure components by chromatographical means. All synthetic intermediates were characterized by TLC, elemental analysis, and UV and ^{1}H-NMR spectra.

1. Introduction. – Establishment of an antiviral state in cells results from a multitude of biochemical changes induced by interferons and involving several proteins in a cascade of reactions [2–4]. One of the proteins is the enzyme 2′–5′A synthetase, which is activated upon binding to double-stranded RNA [5] and converts then ATP into a series of (2′–5′)-linked adenylate oligonucleotides containing a 5′-terminal triphosphate function [6]. Such oligomers carrying a 5′-di- or 5′-triphosphate and consisting of three or more monomer units bind to, and subsequently reversibly activate, an endogenous or sometimes interferon-induced endoribonuclease [7] [8]. The activated endoribonuclease, RNase L, cleaves then messenger and ribosomal RNA's [9], resulting in inhibition of translation. This effect, however, is only transitory, since the 2′–5′A molecules are rapidly cleaved by cellular phosphodiesterases [10] leading to loss of antiviral activity. In order to suppress the digestion of the 2′–5′A oligomers, several synthetic modifications of the native structure were accomplished at the aglycone [11–20], the sugar [21–37], and the phosphate moiety [38–45]. It is well established, mainly due to the pioneering work of *Eckstein* [46], that phosphorothioate analogues of oligonucleotides are valuable models to study certain stereochemical aspects of enzyme-catalyzed phosphoryl and nucleotidyl transfer reactions.

The first synthesis of phosphorothioate analogues of (2′–5′)oligoadenylates were performed by *Nelson*, *Bach*, and *Verheyden* [42] applying the phosphite triester approach in conjunction with sulfur oxidation. Formation of chiral phosphorothioate functions led to diastereoisomeric mixtures of which the dimer cores could be separated chromato-

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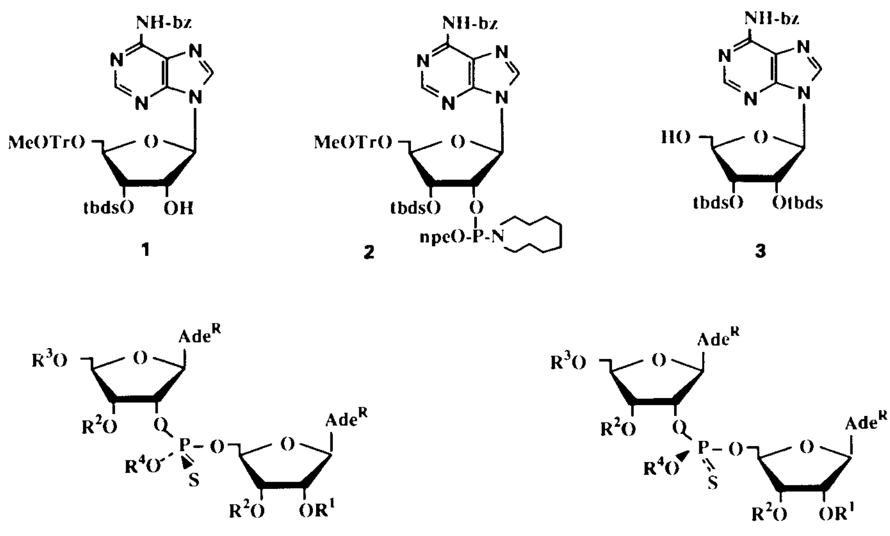
graphically, whereas the trimer cores were studied in form of the corresponding diastereoisomeric pairs. It was found [47] that the configuration at the P-atoms markedly affects the biochemical and biological properties of the phosphorothioate analogues of the 2′–5′A core, revealing also a significantly higher enzymatic stability in the (*PS*)- over the (*PR*)-configuration. Furthermore, the synthesis of the two individual diastereoisomeric 2′–5′A trimer cores containing only one phosphorothioate linkage between the middle and the 2′-terminal unit was reported [48].

Finally, we successfully achieved the syntheses and separation of the two 2′–5′A (*PR*)- and (*PS*)-dimer cores **6a** and **6b**, respectively, as well as the four diastereoisomeric 2′–5′A (*PR,PR*)-, (*PR,PS*)-, (*PS,PR*)-, and (*PS,PS*)-trimer cores **11a**, **12a**, **11b**, and **12b**, respectively, and accomplished the configurational assignment of the new chiral centers by HPLC, charge separation, ^{31}P-NMR spectra and enzymatic hydrolyses [49] [50]. It was also demonstrated that the configuration of the internucleotidic phosphorothioate linkages does not affect the binding to RNase L but markedly controls the activation process [51]. Activation decreases in the order (*PR,PR*) > (*PS,PR*) > (*PR,PS*), whereas the (*PS,PS*)-2′–5′A trimer core and its 5′-monophosphate can be considered as effective inhibitors, which bind strongly to RNase L, but are unable to activate the enzyme up to concentrations as high as 10^{-3} and 10^{-5} M, respectively. We now describe the detailed synthesis of the dimers **6a, b** and the trimers **11a, b** and **12a, b** as well as of the corresponding 5′-monophosphates **15a, b** and **16a, b** on a preparative scale.

2. Syntheses. – The chemical syntheses of the pure diastereoisomeric 2′–5′A dimer and trimer cores on a preparative scale in solution were achieved by the phosphoramidite

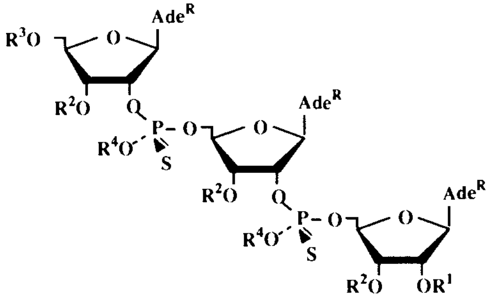
Scheme



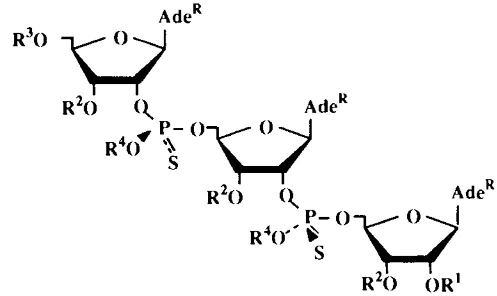
	R	R^1	R^2	R^3	R^4
4a	bz	tbds	tbds	MeOTr	npe
5a	bz	tbds	tbds	H	npe
6a	H	H	H	H	NH_4

	R	R^1	R^2	R^3	R^4
4b	bz	tbds	tbds	MeOTr	npe
5b	bz	tbds	tbds	H	npe
6b	H	H	H	H	NH_4

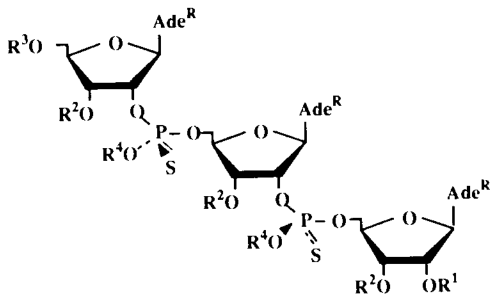
Scheme (cont.)



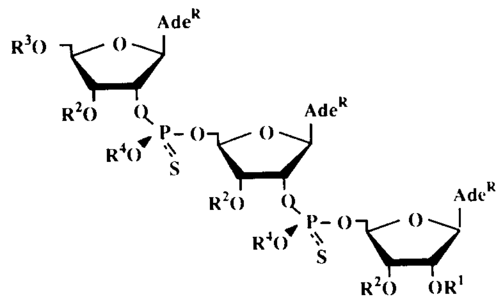
	R	R¹	R²	R³	R⁴
7a	bz	tbds	tbds	MeOTr	npe
8a	bz	tbds	tbds	H	npe
11a	H	H	H	H	NH_4
13a	bz	tbds	tbds	X	npe
15a	H	H	H	$PO_3(NH_4)_2$	NH_4



	R	R¹	R²	R³	R⁴
7b	bz	tbds	tbds	MeOTr	npe
8b	bz	tbds	tbds	H	npe
11b	H	H	H	H	NH_4
13b	bz	tbds	tbds	X	npe
15b	H	H	H	$PO_3(NH_4)_2$	NH_4

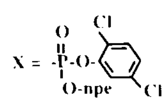


	R	R¹	R²	R³	R⁴
9a	bz	tbds	tbds	MeOTr	npe
10a	bz	tbds	tbds	H	npe
12a	H	H	H	H	NH_4
14a	bz	tbds	tbds	X	npe
16a	H	H	H	$PO_3(NH_4)_2$	NH_4



	R	R¹	R²	R³	R⁴
9b	bz	tbds	tbds	MeOTr	npe
10b	bz	tbds	tbds	H	npe
12b	H	H	H	H	NH_4
14b	bz	tbds	tbds	X	npe
16b	H	H	H	$PO_3(NH_4)_2$	NH_4

bz = benzoyl
tbds = (*tert*-butyl)dimethylsilyl
MeOTr = monomethoxytrityl
npe = 2-(4-nitrophenyl)ethyl



approach, sulfur oxidation, and subsequent separation of the isomers by silica-gel chromatography. In the first step, N^6-benzoyl-3′-*O*-[(*tert*-butyl)dimethylsilyl]-5′-*O*-(monomethoxytrityl)adenosine (**1**) [52] was converted by chloro[2-(4-nitrophenyl)ethoxy](octahydro-1*H*-azonin-1-yl)phosphane [53] into the corresponding phosphoramidite **2** in 97% yield. This building block was then condensed with N^6-benzoyl-2′,3′-bis-*O*-[(*tert*-butyl)-

dimethylsilyl]adenosine (**3**) in MeCN in presence of 3-nitro-1*H*-1,2,4-triazole and subsequent oxidation by sulfur in pyridine to give the corresponding fully protected 2′–5′A phosphorothioate dimers **4a** and **4b** as a diastereoisomer mixture. These isomers could be separated by silica-gel chromatography on prep. TLC plates using CH_2Cl_2/hexane/AcOEt 1:1:1. The faster running component **4a** was obtained in 42% yield and turned out to possess the (*PR*)-configuration, whereas the slower moving diastereoisomer **4b** with (*PS*)-configuration was isolated in 28% yield. Detritylation of these compounds using 2% 4-toluenesulfonic acid in CH_2Cl_2/MeOH 4:1 afforded in yields of *ca.* 90% the 5′-OH components **5a** and **5b**, respectively, which were then separately condensed again with the phosphoramidite **2** in an analogous manner. Subsequent sulfur oxidation yielded the two new diastereoisomeric pairs **7a**(*PR,PR*)/**7b**(*PS,PR*) from **5a** and **9a**(*PR,PS*)/**9b**(*PS,PS*) from **5b**, respectively. Separation into the pure components was achieved again by prep. TLC on silica gel to give, always in higher yields, the faster moving isomer which has in all cases the (*PR*)-configuration.

The four diastereoisomeric 2′–5′A trimer cores **7a**, **7b**, **9a**, and **9b** were then detritylated in the usual manner to the corresponding 5′-OH derivatives **8a**, **8b**, **10a**, and **10b**, respectively, which were furthermore converted by reaction with (2,5-dichlorophenyl)-phosphoditriazolide and subsequent treatment with 2-(4-nitrophenyl)ethanol into their fully protected 5′-*O*-phosphotriesters **13a**, **13b**, **14a**, and **14b** in yields of 70–80%.

The final deblocking of the various protecting groups from the dimers **5a** and **5b** and from the trimers **9a**, **9b**, **10a**, and **10b** was achieved by subsequent treatment *1*) with DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) in abs. pyridine to eliminate the 2-(4-nitrophenyl)-ethyl group, *2*) with Bu_4NF in THF for removal of the (*tert*-butyl)dimethylsilyl groups, and *3*) with conc. ammonia to cleave the benzoyl groups. The resulting dimers **6a** and **6b** and the corresponding trimers **11a**, **11b**, **12a**, and **12b**, respectively, were isolated and purified by *DEAE-Sephadex* column chromatography using a linear gradient of $(Et_3NH)HCO_3$ and subsequent purification by paper chromatography in i-PrOH/conc. NH_3/H_2O 7:1:2.

The deprotection of the fully protected 5′-*O*-phosphoryl trimers **13a**, **13b**, **14a**, and **14b** afforded one additional step: removal of the 2,5-dichlorophenoxy group by use of triethylammonium 4-nitrobenzaldehyde oximate was followed by the described treatment with DBU, Bu_4NF, and NH_3 to give, after the purification procedures, the corresponding 5′-*O*-monophosphate phosphorothioate analogues **15a**, **15b**, **16a**, and **16b** of (2′–5′)adenylate trimer in 68–74% isolated yields.

3. Physical Data. – The characterization of the protected nucleotides was performed by C,H,N analyses, UV spectra, ^{1}H- and ^{31}P-NMR spectra, and their chromatographic behaviour (see *Table*). The ^{1}H-NMR spectra were difficult to analyse due to many overlapping signals; therefore, only some distinct signals like those of the aglycones, the anomeric protons, and the MeO group are reported to help finding the anticipated reaction product during the isolation procedures. The ^{31}P-NMR data and the HPLC retention times of the blocked oligonucleotides were already reported elsewhere [50].

Table. *Physical Data of Phosphorothioate Analogues of (2′–5′) Adenylate Dimer and Trimer*

		UV Spectra (MeOH)		^{1}H-NMR Spectra ($CDCl_3$, δ [ppm])			^{31}P-NMR Spectra ($CDCl_3$)	TLC R_f
		λ_{max} [nm]	lg ε	H–C(1′)	H–C(8)	H–C(2)		
2		230	4.47	6.12 (*d*)			155.27	
		277	4.50				154.01	
4a	(*PR*)	231	4.66	6.31 (*d*), 5.87 (*d*)	8.68 (*s*), 8.60 (*s*)	8.19 (*s*), 8.17 (*s*)	69.81	0.54[a])
		277	4.70					
b	(*PS*)	231	4.69	6.29 (*d*), 5.94 (*d*)	8.72 (*s*), 8.62 (*s*)	8.26 (*s*), 8.18 (*s*)	69.22	0.46[a])
		277	4.70					
5a	(*PR*)	278	4.70	6.07 (*d*), 5.94 (*d*)	8.82 (*s*), 8.75 (*s*)	8.25 (*s*), 8.03 (*s*)		0.27[a]), 0.15[b])
b	(*PS*)	278	4.70	6.13 (*d*), 5.90 (*d*)	8.74 (*s*), 8.73 (*s*)	8.26 (*s*), 8.24 (*s*)		0.30[a]), 0.18[b])
6a	(*PR*)	258[c])		6.14 (*d*), 5.81 (*d*)[d])	8.22 (*s*, 3H)[d])	7.79 (*s*)[d])	57.63[e])	0.29[b])
b	(*PS*)	258[c])		6.17 (*d*), 5.92 (*d*)[d])	8.32 (*s*, 2H)[d])	8.24 (*s*), 7.98 (*s*)[d])	56.13[e])	0.30[b])
7a	(*PR,PR*)	230	4.78	6.23 (*d*), 6.08 (*d*), 5.84 (*d*)	8.69 (*s*), 8.58 (*s*), 8.55 (*s*)	8.20 (*s*), 8.11 (*s*), 8.01 (*s*)	69.80, 69.47	0.58[b])
		278	4.90					
b	(*PS,PR*)	230	4.78	6.22 (*d*), 6.17 (*d*), 5.84 (*d*)	8.67 (*s*), 8.60 (*s*), 8.57 (*s*)	8.23 (*m*, 3H)		0.42[b])
		278	4.89					
8a	(*PR,PR*)	231 (sh)	4.64	6.11 (*d*), 6.03 (*d*), 5.84 (*d*)	8.67 (*s*), 8.65 (*s*), 8.55 (*s*)	8.09 (*m*, 3H)		0.24[b])
		278	4.87					
b	(*PS,PR*)	231 (sh)	4.72	6.19 (*d*), 6.10 (*d*), 5.86 (*d*)	8.70 (*s*), 8.65 (*s*), 8.54 (*s*)	8.20 (*m*, 3H)		0.16[a]), 0.30[b])
		277	4.87					
9a	(*PR,PS*)	230	4.78	6.27 (*d*), 6.13 (*d*), 5.93 (*d*)	8.71 (*s*), 8.61 (*s*), 8.60 (*s*)	8.21 (*s*), 8.13 (*m*, 2H)		0.54[b])
		278	4.90					
b	(*PS,PS*)	230	4.78	6.27 (*d*), 6.22 (*d*), 5.90 (*d*)	8.71 (*s*), 8.64 (*s*), 8.61 (*s*)	8.27 (*s*), 8.22 (*s*), 8.19 (*s*)		0.43[b])
		278	4.90					
10a	(*PR,PS*)	231 (sh)	4.70	6.17 (*d*), 6.06 (*d*), 5.90 (*d*)	8.71 (*s*), 8.67 (*s*), 8.62 (*s*)	8.22 (*m*, 3H)		0.12[b])
		277	4.87					
b	(*PS,PS*)	231 (sh)	4.66	6.18 (*d*), 6.10 (*d*), 5.91 (*d*)	8.73 (*s*), 8.69 (*s*), 8.65 (*s*)	8.25 (*s*), 8.22 (*s*), 8.20 (*s*)		0.17[b])
		278	4.86					
11a	(*PR,PS*)	258[c])		6.04 (*d*), 5.92 (*d*), 5.78 (*d*)[d])	8.33 (*s*), 8.16 (*s*), 8.13 (*s*)[d])	8.09 (*s*), 7.90 (*s*), 7.70 (*s*)[d])	57.45, 57.71[e])	0.52[f])
b	(*PS,PR*)	258[c])		6.09 (*d*), 6.00 (*d*), 5.80 (*d*)[d])	8.23 (*s*), 8.15 (*s*, 2H)[d])	8.05 (*s*), 7.90 (*s*), 7.70 (*s*)[d])	56.62, 57.55[e])	0.52[f])
12a	(*PR,PS*)	258[c])		6.04 (*d*), 5.91 (*d*), 5.81 (*d*)[d])	8.27 (*s*), 8.16 (*s*), 8.09 (*s*)	8.05 (*s*), 7.96 (*s*), 7.68 (*s*)[d])	57.54, 56.34[e])	0.52[f])
b	(*PS,PS*)	258[c])		6.05 (*d*), 5.95 (*d*), 5.82 (*d*)[d])	8.15 (*s*), 8.10 (*s*, 2H)[d])	8.04 (*s*), 7.92 (*s*), 7.78 (*s*)[d])	56.50, 56.26[e])	0.52[f])
15a	(*PR,PR*)	258[c])		6.04 (*d*), 5.92 (*d*), 5.74 (*d*)[d])	8.31 (*s*), 8.18 (*s*), 8.12 (*s*)[d])	7.98 (*s*), 7.85 (*s*), 7.80 (*s*)[d])		0.18[g])
b	(*PS,PR*)	258[c])		6.12 (*d*), 5.95 (*d*), 5.76 (*d*)[d])	8.26 (*s*), 8.21 (*s*), 8.11 (*s*)[d])	7.99 (*s*), 7.94 (*s*), 7.86 (*s*)[d])		0.18[g])
16a	(*PR,PS*)	258[c])		6.03 (*d*), 5.92 (*d*), 5.80 (*d*)[d])	8.27 (*s*), 8.22 (*s*), 8.15 (*s*)[d])	8.04 (*s*), 7.93 (*s*), 7.81 (*s*)[d])		0.18[g])
b	(*PS,PS*)	258[c])		6.09 (*d*), 5.94 (*d*), 5.81 (*d*)[d])	8.41 (*s*), 8.26 (*s*), 8.14 (*s*)[d])	8.07 (*s*), 8.02 (*s*), 7.89 (*s*)[d])		0.18[g])

[a]) SiO_2, CH_2Cl_2/AcOEt/hexane 1:1:1. [b]) SiO_2, CH_2Cl_2/AcOEt/hexane 1:1:0.5. [c]) In H_2O. [d]) In D_2O. [e]) In D_2O. [f]) Cellulose, i-PrOH/conc. NH_3 soln./H_2O 6:1:3. [g]) Cellulose, i-PrOH/conc. NH_3 soln./H_2O 55:10:35.

Experimental Part

General. TLC: precoated silica-gel thin-layer sheets *F 1500 LS 254* and cellulose thin-layer sheets *F 1440* from *Schleicher & Schüll.* Prep. TLC: silica gel *60 PF_{254}* (*Merck*). Prep. column chromatography (CC): silica gel (*Merck 60*, 0.063–0.2 mesh). Paper chromatography: PC sheets (58 × 60 cm) from *Schleicher & Schüll.* Ion-exchange chromatography: *DEAE Sephadex A-25* (*Pharmacia*). UV/VIS: *Uvikon 820* (*Kontron*); λ_{max} in nm (lg ε). ^{1}H-NMR: *Bruker WM 250*; δ in ppm rel. to $CHCl_3$, deblocked compounds in D_2O.

1. *N^6-Benzoyl-3'-O-[(*tert*-butyl)dimethylsilyl]-5'-O-(monomethoxytrityl)adenosine 2'-[2-(4-Nitrophenyl)ethyl N,N-Octamethylenephosphoramidite]* (**2**). To a soln. of 0.758 g (1 mmol) of **1** [52] and 0.52 g (4 mmol) of Et(i-Pr_2)N in CH_2Cl_2 (5 ml), 0.8 g (2.22 mmol) of chloro[2-(4-nitrophenyl)ethoxy](octahydro-1*H*-azonin-1-yl)phosphane [53] was added dropwise under N_2. After stirring at r.t. for 2 h, the mixture was treated with sat. aq. $NaHCO_3$ soln. (50 ml) and then the product isolated by extraction with AcOEt (2 × 50 ml). The org. layer was washed with sat. NaCl soln., dried (Na_2SO_4), and evaporated. The residue was dissolved in AcOEt/Et_3N 95:5 and submitted to CC (silica gel, 10 × 2 cm, equilibration with AcOEt/Et_3N 9:1 and 95:5, elution with AcOEt/Et_3N 95:5). The product fractions were evaporated, coevaporated with CH_2Cl_2 (5 × 10 ml), and dried at 40°/h.v.: 1.05 g (97%) of **2**. ^{31}P-NMR ($CDCl_3$): 155.27, 154.01. Anal. calc. for $C_{59}H_{70}N_7O_9PSi \cdot 1\ H_2O$ (1098.3): C 64.52, H 6.60, N 8.92; found: C 63.93, H 6.85, N 8.62.

2. *(*PR*)- and (*PS*)-N^6-Benzoyl-3'-O-[(*tert*-butyl)dimethylsilyl]-5'-O-(monomethoxytrityl)-P-thioadenylyl-{2'-{O^P-[2-(4-nitrophenyl)ethyl]}→5'}-N^6-benzoyl-2',3'-bis-O-[(*tert*-butyl)dimethylsilyl]adenosine* (**4a** and **4b**, resp.). Phosphoramidite **2** (1.09 g, 1 mmol) and N^6-benzoyl-2',3'-bis-*O*-[(*tert*-butyl)dimethylsilyl]adenosine (**3**; 0.478 g, 0.7 mmol) were dried overnight at 40°/h.v. The mixture was then dissolved in dry MeCN (6 ml), 3-nitro-1*H*-1,2,4-triazole (0.285 g, 2.5 mmol) [54] was added and the mixture stirred at r.t. for 3 h (TLC: complete conversion). Pyridine (6 ml) and sulfur (0.5 g, 15.6 mmol) were added, and after stirring at r.t. for 20 h, the mixture was extracted with $CHCl_3$ (300 ml), the org. layer washed with sat. NaCl soln. (2 × 200 ml), dried (Na_2SO_4), and evaporated, and the residue coevaporated with toluene (2 × 20 ml). The residue was dissolved in $CHCl_3$ and submitted to CC (silica gel, 15 × 2.5 cm, $CHCl_3$ (1 l)). The product fractions containing both (*PR*)- and (*PS*)-isomers were evaporated, and their separation was achieved by prep. TLC (silica gel, CH_2Cl_2/AcOEt/hexane 1:1:1): 0.47 g (42%; TLC (same system): R_f 0.54) of **4a** and 0.31 g (28%; TLC: R_f 0.46) of **4b**, both as colourless amorphous powders after drying at 40°.

4a (*PR*): ^{31}P-NMR ($CDCl_3$): 69.84. Anal. calc. for $C_{80}H_{98}N_{10}O_{14}PSSi_3 \cdot H_2O$ (1589.0): C 59.89, H 6.28, N 9.61; found: C 59.89, H 6.32, N 9.21.

4b (*PS*): ^{31}P-NMR ($CDCl_3$): 69.22. Anal. calc. for $C_{80}H_{98}N_{10}O_{14}PSSi_3 \cdot H_2O$ (1589.0): C 59.89, H 6.28, N 9.61; found: C 59.91, H 6.41, N 9.28.

3. *(*PR*)- and (*PS*)-N^6-Benzoyl-3'-O-[(*tert*-butyl)dimethylsilyl]-P-thioadenylyl-{2'-{O^P-[2-(4-nitrophenyl)ethyl]}→5'}-N^6-benzoyl-2',3'-bis-O-[(*tert*-butyl)dimethylsilyl]adenosine* (**5a** and **5b**, resp.). Dimers **4a** or **4b** (0.258 g, 0.164 mmol) was treated with 2% TsOH in CH_2Cl_2/MeOH 4:1 (3.2 ml) at r.t. for 40 min. The mixture was taken up in $CHCl_3$ (50 ml), washed with phosphate buffer pH 7.5 (2 × 20 ml), and evaporated to a foam. The residue was purified by CC (silica gel, 10 × 2.5 cm, first $CHCl_3$, then $CHCl_3$/MeOH 100:0.5 and 100:1, resp.). The product fractions were evaporated and dried at 40°/h.v.: 0.198 g (92%) of **5a** and 0.192 g (89%) of **5b**, resp.

5a (*PR*): TLC (CH_2Cl_2/AcOEt/hexane 1:1:1): R_f 0.27. Anal. calc. for $C_{60}H_{82}N_{11}O_{13}PSSi_3 \cdot H_2O$ (1330.7): C 54.15, H 6.36, N 11.57; found: C 53.78, H 6.44, N 10.72.

5b (*PS*): TLC (CH_2Cl_2/AcOEt/hexane 1:1:1): R_f 0.30. Anal. calc. for $C_{60}H_{82}N_{11}O_{13}PSSi_3$ (1312.7): C 54.90, H 6.29, N 11.73; found: C 54.90, H 6.20, N 11.45.

4. *(*PR*)- and (*PS*)-P-Thioadenylyl-(2'–5')-adenosine* (diammonium salts; **6a** and **6b**, resp.). In a soln. of 0.5M DBU in dry pyridine (9 ml) was treated 0.039 g (0.03 mmol) of **5a** or **5b** by stirring at r.t. for 2 h. The mixture was neutralized with 1M AcOH in dry pyridine (4.5 ml) and then evaporated. The residue was taken up in 1M Bu_4NF in THF (5 ml), and after stirring at r.t. for 24 h, the mixture was again evaporated. The resulting residue was treated in conc. NH_3 soln. (20 ml) by stirring at r.t. for 48 h. After another evaporation, the residue was taken up in H_2O (50 ml) and washed with $CHCl_3$ (2 × 20 ml) and the H_2O phase applied onto a *DEAE-Sephadex* column *A-25* (60 × 1 cm). Elution was performed with a linear gradient (0.001–0.25M) of (Et_3NH)HCO_3 buffer (pH 7.5). The product fractions were evaporated several times with H_2O. The Et_3NH^+ salt was converted into its NH_4^+ salt by paper chromatography using i-PrOH/NH_3/H_2O 7:1:2 by H_2O and lyophilization to yield 630 *OD* units (A_{260}) of **6a** (87.5%) and 648 *OD* units (A_{260}) of **6b** (90%) as colourless powders, resp. TLC (cellulose, i-PrOH/conc. NH_3 soln./H_2O 7:1:2): R_f (**6a**) 0.29; R_f (**6b**) 0.30.

5. *(*PR*)- and (*PS*)-*N^6*-Benzoyl-3'-*O*-[(*tert*-butyl)dimethylsilyl]-5'-*O*-(monomethoxytrityl)-*P*-thioadenylyl-{2'-{*O^P*-[2-(4-nitrophenyl)ethyl]}→5'}-(*PR*)-*N^6*-benzoyl-3'-*O*-[(*tert*-butyl)dimethylsilyl]-*P*-thioadenylyl-{2'-{*O^P*-[2-(4-nitrophenyl)ethyl]}→5'}-*N^6*-benzoyl-2',3'-bis-*O*-[(*tert*-butyl)dimethylsilyl]adenosine* (**7a** and **7b**, resp.) *and Their (*PR,PS*)- and (*PS,PS*)-Isomers* **9a** *and* **9b**, *resp.* A soln. of **2** (0.449 g, 0.41 mmol), 0.262 g (0.2 mmol) of **5a** or **5b**, and 3-nitro-1*H*-1,2,4-triazole (0.114 g, 1 mmol) in MeCN (3.2 ml) was stirred at r.t. for 3 h. Then, sulfur (0.2 g, 6.25 mmol) and pyridine (0.4 ml) were added, and the mixture was stirred at r.t. for another 20 h. The product was extracted with CH_2Cl_2 (50 ml), the org. phase washed with sat. NaCl soln. (2 × 20 ml), dried (Na_2SO_4), and evaporated. Final co-evaporation was done with toluene to remove pyridine. The crude product was purified by CC (silica gel; 15 × 2 cm, $CHCl_3$/MeOH 50:1) and the two diastereoisomers were then separated by prep. TLC (silica gel; 20 × 20 × 0.2 cm, CH_2Cl_2/AcOEt/hexane 1:1:0.5, 3 times). The product bands were eluted with $CHCl_3$/MeOH 4:1: less polar **7a** (0.218 g, 48%) and more polar **7b** (0.154 g, 34%) as colourless amorphous powders.

7a (*PR,PR*): Anal. calc. for $C_{111}H_{135}N_{17}O_{22}P_2S_2Si_4 \cdot H_2O$ (2315.8): C 57.56, H 5.96, N 10.28; found: C 57.38, H 5.99, N 10.11.

7b (*PS,PR*): Anal. calc. for $C_{111}H_{135}N_{17}O_{22}P_2S_2Si_4 \cdot H_2O$ (2315.8): C 57.56, H 5.96, N 10.28; found: C 57.40, H 5.97, N 10.19.

Condensation of **2** and **5b** according to the same procedure gave the less polar **9a** (0.186 g, 41%) and the more polar **9b** (0.159 g, 35%) in form of colourless solid foams.

9a (*PR,PS*): Anal. calc. for $C_{111}H_{135}N_{17}O_{22}P_2S_2Si_4$ (2297.8): C 58.02, H 5.92, N 10.36; found: C 58.00, N 5.84, N 10.66.

9b (*PS,PS*): Anal. calc. for $C_{111}H_{135}N_{17}O_{22}P_2S_2Si_4 \cdot H_2O$ (2315.8): C 57.56, H 5.96, N 10.28; found: C 57.30, H 5.78, N 10.03.

6. *(*PR*)- and (*PS*)-*N^6*-Benzoyl-3'-*O*-[(*tert*-butyl)dimethylsilyl]-*P*-thioadenylyl-{2'-{*O^P*-[2-(4-nitrophenyl)-ethyl]}→5'}-(*PR*)-*N^6*-benzoyl-3'-*O*-[(*tert*-butyl)dimethylsilyl]-*P*-thioadenylyl-{2'-{*O^P*-[2-(4-nitrophenyl)-ethyl]}→5'}-*N^6*-benzoyl-2',3'-bis-*O*-[(*tert*-butyl)dimethylsilyl]adenosine* (**8a** and **8b**, resp.) *and Their (*PR,PS*)- and (*PS,PS*)-Isomers* **10a** *and* **10b**, *resp.* At r.t., **7a**, **7b**, **9a**, or **9b** (0.149 g, 0.065 mmol) was stirred with 2% TsOH in CH_2Cl_2/MeOH 4:1 (1.5 ml) for 3 h. The soln. was taken up in $CHCl_3$ (50 ml) and washed with H_2O (2 × 25 ml), the $CHCl_3$ phase dried (Na_2SO_4) and evaporated, and the crude product purified by prep. TLC (silica gel; 20 × 20 × 0.2 cm, CH_2Cl_2/AcOEt/hexane 1:1:0.5). The pure product was eluted from the plates with $CHCl_3$/MeOH 1:1 giving in all cases 80% yields.

8a (*PR,PR*): Anal. calc. for $C_{91}H_{119}N_{17}O_{21}P_2S_2Si_4 \cdot H_2O$ (2043.7): C 53.48, H 5.96, N 11.65; found: C 53.22, H 5.95, N 11.51.

8b (*PS,PR*): Anal. calc. for $C_{91}H_{119}N_{17}O_{21}P_2S_2Si_4$ (2025.7): C 53.95, H 5.87, N 11.75; found: C 53.61, H 6.00, N 11.49.

10a (*PR,PS*): Anal. calc. for $C_{91}H_{119}N_{17}O_{21}P_2S_2Si_4$ (2025.7): C 53.95, H 5.87, N 11.75; found: C 53.65, H 5.87, N 11.38.

10b (*PS,PS*): Anal. calc. for $C_{91}H_{119}N_{17}O_{21}P_2S_2Si_4$ (2025.7): C 53.48, H 5.96, N 11.65; found: C 53.18, H 5.88, N 11.50.

7. *(*PR*)- and (*PS*)-*P*-Thioadenylyl-(2'→5')-(*PR*)-*P*-thioadenylyl-(2'→5')-adenosine* (diammonium salts; **11a** and **11b**, resp.) *and Their (*PR,PS*)- and (*PS,PS*)-Isomers* **12a** *and* **12b**, *resp.* A soln. of 38.5 mg (0.0189 mmol) of **8a**, **8b**, **10a**, or **10b** in 0.5M DBU in dry pyridine (7.5 ml) was stirred at r.t. for 20 h. The mixture was neutralized with 1M AcOH in dry pyridine (3.75 ml) and then evaporated. The residue was taken up in 1M Bu_4NF in THF, and after stirring for 48 h, the solvent was evaporated and the residue treated with conc. NH_3 soln. (25 ml) for another 48 h. The solvent was evaporated and the residue dissolved in H_2O (20 ml) and washed with $CHCl_3$ (2 × 10 ml). The aq. phase was put onto a *DEAE Sephadex A-25* column (60 × 1 cm) and the product eluted with a linear gradient (0.001–0.5M) of $(Et_3NH)HCO_3$ buffer (pH 7.5). The product fractions were evaporated and co-evaporated several times with H_2O. The products were further purified by paper chromatography (i-PrOH/conc. NH_3 soln./H_2O 6:1:3) to give, after lyophilisation, the fully deblocked trimers in 75–80% yields as colourless powders.

8. *N^6*-Benzoyl-3'-*O*-[(*tert*-butyl)dimethylsilyl]-5'-*O*-{(2,5-dichlorophenoxy)[2-(4-nitrophenyl)ethoxy]phosphoryl}-*P*-thioadenylyl-{2'-{*O^P*-[2-(4-nitrophenyl)ethyl]}→5'}-*N^6*-benzoyl-3'-*O*-[(*tert*-butyl)dimethylsilyl]-*P*-thioadenylyl-{2'-{*O^P*-[2-(4-nitrophenyl)ethyl]}-5'}-*N^6*-benzoyl-2',3'-di-*O*-[(*tert*-butyl)dimethylsilyl]adenosine* (**13a** (*PR,PR*), **13b** (*PS,PR*), **14b** (*PR,PS*), and **14b** (*PS,PS*)). To a soln. of 1*H*-1,2,4-triazole (0.011 g, 0.16 mmol) and 2,5-dichlorophenyl phosphorodichloridate (0.022 g, 0.078 mmol) in dry pyridine (0.5 ml) was added **8a**, **8b**, **10a**, or **10b** (80.1 mg, 0.049 mmol) in dry pyridine (0.5 ml). After stirring for 30 min, 2-(4-nitrophenyl)ethanol (0.02 g, 0.119 mmol) was added and stirring continued at r.t. for 20 h. The product was extracted with $CHCl_3$ (50 ml), the org. phase washed with H_2O (2 × 20 ml), evaporated, and finally co-evaporated with toluene. The residue

was purified by prep. TLC (silica gel; 20 × 20 × 0.2 cm, CH_2Cl_2/AcOEt/hexane 1:1:1). The product band was eluted with $CHCl_3$/MeOH 7:3 and gave, on evaporation, the trimer 5′-monophosphates in 70–80% yield as colourless amorphous powders.

9. *5′-O-Phosphoryl-*P*-thioadenylyl-(2′→5′)-*P*-thioadenylyl-(2′→5′)-adenosine* (tetraammonium salts; **15a** (*PR,PR*), **15b** (*PS,PR*), **16a** (*PR,PS*), and **16b** (*PS,PS*)). A soln. of 4-nitrobenzaldehydeoxime (0.036 g, 0.216 mmol) in dioxane/Et_3N/H_2O (each 0.5 ml) was stirred for 30 min. Then **13a**, **13b**, **14a**, or **14b** (0.05 g, 0.02 mmol) was added and stirred at r.t. for 4 h. After evaporation and co-evaporation with toluene (2 × 5 ml), the product was purified by prep. TLC (silica gel; 20 × 20 × 0.2 cm, CH_2Cl_2/MeOH 95:5). The product band was eluted with $CHCl_3$/MeOH/Et_3N 5:1:1 and, after evaporation and drying (0.022 g, 0.01 mmol), treated with 0.5M DBU in pyridine (8 ml). After stirring for 24 h, the mixture was neutralized with 1M AcOH in pyridine (4 ml) and again evaporated. The residue was taken up in 1M Bu_4NF in THF (6 ml), and, after stirring at r.t. for 48 h and evaporation, debenzoylation was achieved by treatment with conc. NH_3 soln. (25 ml) for 48 h at r.t. The mixture was evaporated, the residue taken up in H_2O (25 ml) and washed with $CHCl_3$ (2 × 10 ml), and the aq. phase applied onto a *DEAE-Sephadex A-25* column (60 × 1 cm) for elution with a linear gradient (0.001–1M) of $(Et_3NH)HCO_3$ buffer (pH 7.5). The product fractions were evaporated and co-evaporated several times with H_2O. Further purification by paper chromatography (i-PrOH/conc. NH_3 soln./H_2O 55:10:35) gave, after lyophilisation, colourless powders of the trimer 5′-monophosphates in 68–74% yield.

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