

### 35. Nucleotides

Part XXXVIII<sup>1)</sup>

#### 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

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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 <sup>1</sup>H-NMR spectra.

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**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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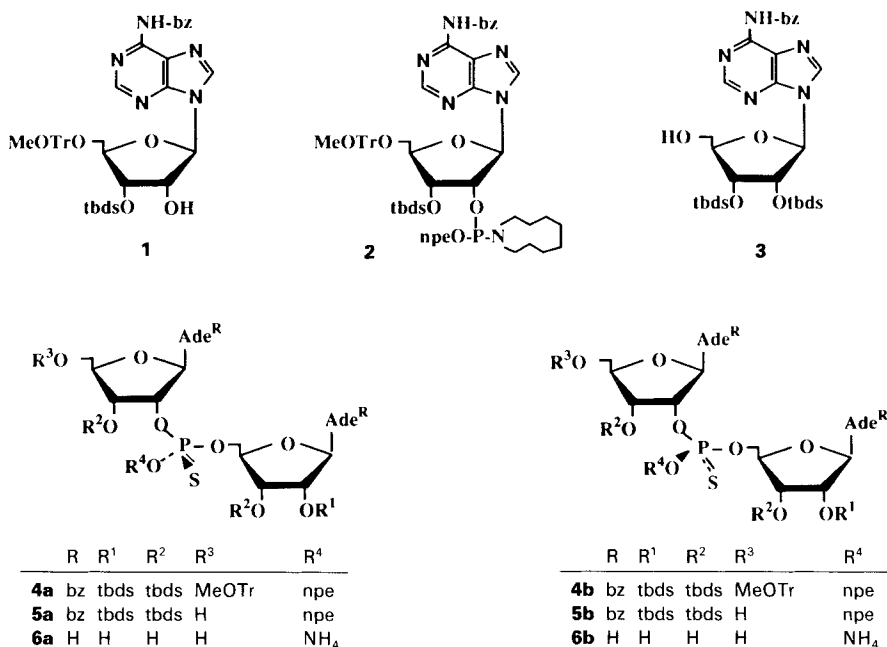
<sup>1)</sup> Part XXXVII: [1].

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].

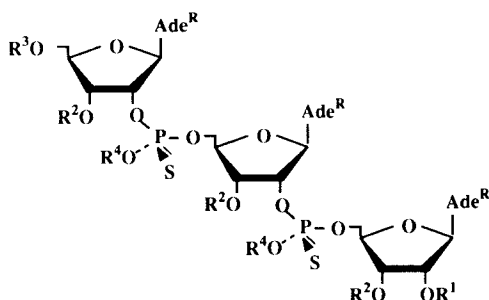
Finally, we successfully achieved the syntheses and separation of the two 2'-5'A (*PR*)- and (*PS*)-dimer cores **6a** and **6b**, respectively, as well as the four diastereoisomeric 2'-5'A (*PR,PR*)-, (*PR,PS*)-, (*PS,PR*)-, and (*PS,PS*)-trimer cores **11a**, **12a**, **11b**, and **12b**, respectively, and accomplished the configurational assignment of the new chiral centers by HPLC, charge separation, <sup>31</sup>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<sup>-3</sup> and 10<sup>-5</sup> 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

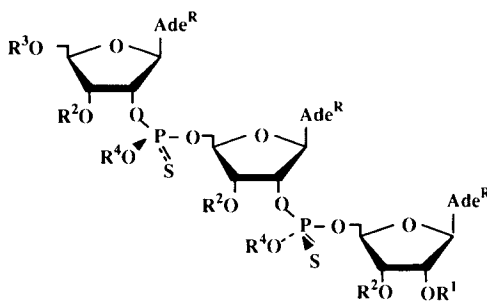
Scheme



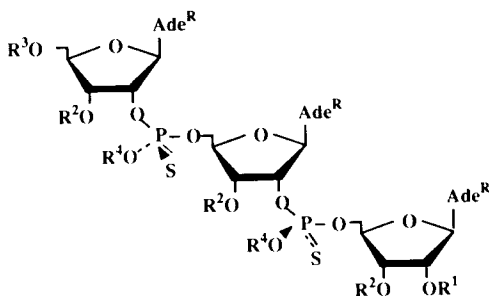
Scheme (cont.)



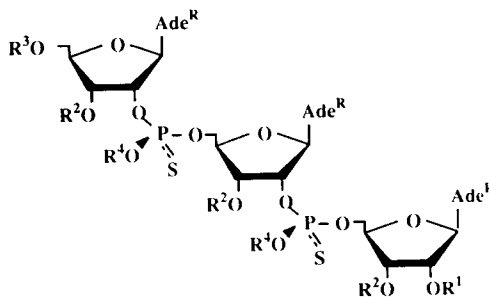
	R	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>
<b>7a</b>	bz	tbds	tbds	MeOTr	npe
<b>8a</b>	bz	tbds	tbds	H	npe
<b>11a</b>	H	H	H	H	NH <sub>4</sub>
<b>13a</b>	bz	tbds	tbds	X	npe
<b>15a</b>	H	H	H	PO <sub>3</sub> (NH <sub>4</sub> ) <sub>2</sub>	NH <sub>4</sub>



	R	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>
<b>7b</b>	bz	tbds	tbds	MeOTr	npe
<b>8b</b>	bz	tbds	tbds	H	npe
<b>11b</b>	H	H	H	H	NH <sub>4</sub>
<b>13b</b>	bz	tbds	tbds	X	npe
<b>15b</b>	H	H	H	PO <sub>3</sub> (NH <sub>4</sub> ) <sub>2</sub>	NH <sub>4</sub>



	R	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>
<b>9a</b>	bz	tbds	tbds	MeOTr	npe
<b>10a</b>	bz	tbds	tbds	H	npe
<b>12a</b>	H	H	H	H	NH <sub>4</sub>
<b>14a</b>	bz	tbds	tbds	X	npe
<b>16a</b>	H	H	H	PO <sub>3</sub> (NH <sub>4</sub> ) <sub>2</sub>	NH <sub>4</sub>



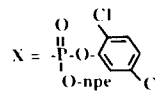
	R	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>
<b>9b</b>	bz	tbds	tbds	MeOTr	npe
<b>10b</b>	bz	tbds	tbds	H	npe
<b>12b</b>	H	H	H	H	NH <sub>4</sub>
<b>14b</b>	bz	tbds	tbds	X	npe
<b>16b</b>	H	H	H	PO <sub>3</sub> (NH <sub>4</sub> ) <sub>2</sub>	NH <sub>4</sub>

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*<sup>6</sup>-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*<sup>6</sup>-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<sub>2</sub>Cl<sub>2</sub>/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<sub>2</sub>Cl<sub>2</sub>/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)-phosphoditriazolidine 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<sub>4</sub>NF 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<sub>3</sub>NH)HCO<sub>3</sub> and subsequent purification by paper chromatography in *i*-PrOH/conc. NH<sub>3</sub>/H<sub>2</sub>O 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<sub>4</sub>NF, and NH<sub>3</sub> 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, <sup>1</sup>H- and <sup>31</sup>P-NMR spectra, and their chromatographic behaviour (see *Table*). The <sup>1</sup>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 <sup>31</sup>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') Adenylylate Dimer and Trimer

	UV Spectra (MeOH)		<sup>1</sup> H-NMR Spectra (CDCl <sub>3</sub> , δ [ppm])		<sup>31</sup> P-NMR Spectra (CDCl <sub>3</sub> )	TLC R <sub>f</sub>
	λ <sub>max</sub> [nm]	lgε	H-C(1')	H-C(8)	H-C(2)	
<b>2</b>	230	4.47	6.12 (d)		155.27	
	277	4.50			154.01	
<b>4a</b> (PR)	231	4.66	6.31 (d), 5.87 (d)	8.68 (s), 8.60 (s)	69.81	0.54 <sup>a)</sup>
	277	4.70				
<b>b</b> (PS)	231	4.69	6.29 (d), 5.94 (d)	8.72 (s), 8.62 (s)	69.22	0.46 <sup>a)</sup>
	277	4.70				
<b>5a</b> (PR)	278	4.70	6.07 (d), 5.94 (d)	8.82 (s), 8.75 (s)		0.27 <sup>a)</sup> , 0.15 <sup>b)</sup>
<b>b</b> (PS)	278	4.70	6.13 (d), 5.90 (d)	8.74 (s), 8.73 (s)		0.30 <sup>a)</sup> , 0.18 <sup>b)</sup>
<b>6a</b> (PR)	258 <sup>c)</sup>		6.14 (d), 5.81 (d) <sup>d)</sup>	8.22 (s, 3H) <sup>d)</sup>	57.63 <sup>c)</sup>	0.29 <sup>b)</sup>
<b>b</b> (PS)	258 <sup>c)</sup>		6.17 (d), 5.92 (d) <sup>d)</sup>	8.32 (s, 2H) <sup>d)</sup>	56.13 <sup>c)</sup>	0.30 <sup>b)</sup>
<b>7a</b> (PR, PR)	230	4.78	6.23 (d), 6.08 (d), 5.84 (d)	8.69 (s), 8.58 (s), 8.55 (s)	69.80, 69.47	0.58 <sup>b)</sup>
	278	4.90				
<b>b</b> (PS, PR)	230	4.78	6.22 (d), 6.17 (d), 5.84 (d)	8.67 (s), 8.60 (s), 8.57 (s)		0.42 <sup>b)</sup>
	278	4.89			8.23 (m, 3H)	
<b>8a</b> (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 <sup>b)</sup>
	278	4.87				
<b>b</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 <sup>a)</sup> , 0.30 <sup>b)</sup>
	277	4.87				
<b>9a</b> (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 <sup>b)</sup>
	278	4.90				
<b>b</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 <sup>b)</sup>
	278	4.90				
<b>10a</b> (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 <sup>b)</sup>
	277	4.87				
<b>b</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 <sup>b)</sup>
	278	4.86				
<b>11a</b> (PR, PS)	258 <sup>c)</sup>		6.04 (d), 5.92 (d), 5.78 (d) <sup>d)</sup>	8.33 (s), 8.16 (s), 8.13 (s) <sup>d)</sup>	57.45, 57.71 <sup>c)</sup>	0.52 <sup>f)</sup>
<b>b</b> (PS, PR)	258 <sup>c)</sup>		6.09 (d), 6.00 (d), 5.80 (d) <sup>d)</sup>	8.23 (s), 8.15 (s, 2H) <sup>d)</sup>	56.62, 57.55 <sup>c)</sup>	0.52 <sup>f)</sup>
<b>12a</b> (PR, PS)	258 <sup>c)</sup>		6.04 (d), 5.91 (d), 5.81 (d) <sup>d)</sup>	8.27 (s), 8.16 (s), 8.09 (s)	57.54, 56.34 <sup>c)</sup>	0.52 <sup>f)</sup>
<b>b</b> (PS, PS)	258 <sup>c)</sup>		6.05 (d), 5.95 (d), 5.82 (d) <sup>d)</sup>	8.15 (s), 8.10 (s, 2H) <sup>d)</sup>	56.50, 56.26 <sup>c)</sup>	0.52 <sup>f)</sup>
<b>15a</b> (PR, PR)	258 <sup>c)</sup>		6.04 (d), 5.92 (d), 5.74 (d) <sup>d)</sup>	8.31 (s), 8.18 (s), 8.12 (s) <sup>d)</sup>	7.98 (s), 7.85 (s), 7.80 (s) <sup>d)</sup>	0.18 <sup>g)</sup>
<b>b</b> (PS, PR)	258 <sup>c)</sup>		6.12 (d), 5.95 (d), 5.76 (d) <sup>d)</sup>	8.26 (s), 8.21 (s), 8.11 (s) <sup>d)</sup>	7.99 (s), 7.94 (s), 7.86 (s) <sup>d)</sup>	0.18 <sup>g)</sup>
<b>16a</b> (PR, PS)	258 <sup>c)</sup>		6.03 (d), 5.92 (d), 5.80 (d) <sup>d)</sup>	8.27 (s), 8.22 (s), 8.15 (s) <sup>d)</sup>	8.04 (s), 7.93 (s), 7.81 (s) <sup>d)</sup>	0.18 <sup>g)</sup>
<b>b</b> (PS, PS)	258 <sup>c)</sup>		6.09 (d), 5.94 (d), 5.81 (d) <sup>d)</sup>	8.41 (s), 8.26 (s), 8.14 (s) <sup>d)</sup>	8.07 (s), 8.02 (s), 7.89 (s) <sup>d)</sup>	0.18 <sup>g)</sup>

<sup>a)</sup> SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/AcOEt/hexane 1:1:1.<sup>b)</sup> SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/AcOEt/hexane 1:1:0.5.<sup>c)</sup> In H<sub>2</sub>O.<sup>d)</sup> In D<sub>2</sub>O.<sup>e)</sup> In D<sub>2</sub>O.<sup>f)</sup> Cellulose, i-PrOH/conc. NH<sub>3</sub> soln./H<sub>2</sub>O 6:1:3.<sup>g)</sup> Cellulose, i-PrOH/conc. NH<sub>3</sub> soln./H<sub>2</sub>O 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<sub>254</sub>* (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);  $\lambda_{\max}$  in nm (lg  $\epsilon$ ).  $^1\text{H-NMR}$ : *Bruker WM 250*;  $\delta$  in ppm rel. to  $\text{CHCl}_3$ , deblocked compounds in  $\text{D}_2\text{O}$ .

1.  $\text{N}^6$ -Benzoyl-3'-O-[(*tert*-butyl)dimethylsilyl]-5'-O-(monomethoxytrityl)adenosine 2'-[2-(4-Nitrophenyl)-ethyl]  $\text{N}_3$ , $\text{N}$ -Octamethylenephosphoramidite (**2**). To a soln. of 0.758 g (1 mmol) of **1** [52] and 0.52 g (4 mmol) of  $\text{Et}(\text{i-Pr}_2)\text{N}$  in  $\text{CH}_2\text{Cl}_2$  (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  $\text{N}_2$ . After stirring at r.t. for 2 h, the mixture was treated with sat. aq.  $\text{NaHCO}_3$  soln. (50 ml) and then the product isolated by extraction with  $\text{AcOEt}$  ( $2 \times 50$  ml). The org. layer was washed with sat.  $\text{NaCl}$  soln., dried ( $\text{Na}_2\text{SO}_4$ ), and evaporated. The residue was dissolved in  $\text{AcOEt}/\text{Et}_3\text{N}$  95:5 and submitted to CC (silica gel,  $10 \times 2$  cm, equilibration with  $\text{AcOEt}/\text{Et}_3\text{N}$  9:1 and 95:5, elution with  $\text{AcOEt}/\text{Et}_3\text{N}$  95:5). The product fractions were evaporated, coevaporated with  $\text{CH}_2\text{Cl}_2$  ( $5 \times 10$  ml), and dried at  $40^\circ/\text{h.v.}$ : 1.05 g (97%) of **2**.  $^{31}\text{P-NMR}$  ( $\text{CDCl}_3$ ): 155.27, 154.01. Anal. calc. for  $\text{C}_{59}\text{H}_{70}\text{N}_7\text{O}_9\text{PSi} \cdot 1 \text{ H}_2\text{O}$  (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*)- $\text{N}^6$ -Benzoyl-3'-O-[(*tert*-butyl)dimethylsilyl]-5'-O-(monomethoxytrityl)-P-thioadenylyl-{2'-{O<sup>p</sup>-[2-(4-nitrophenyl)ethyl]}→5'}- $\text{N}^6$ -benzoyl-2',3'-bis-O-[(*tert*-butyl)dimethylsilyl]adenosine (**4a** and **4b**, resp.). Phosphoramidite **2** (1.09 g, 1 mmol) and  $\text{N}^6$ -benzoyl-2',3'-bis-O-[(*tert*-butyl)dimethylsilyl]adenosine (**3**; 0.478 g, 0.7 mmol) were dried overnight at  $40^\circ/\text{h.v.}$  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  $\text{CHCl}_3$  (300 ml), the org. layer washed with sat.  $\text{NaCl}$  soln. ( $2 \times 200$  ml), dried ( $\text{Na}_2\text{SO}_4$ ), and evaporated, and the residue coevaporated with toluene ( $2 \times 20$  ml). The residue was dissolved in  $\text{CHCl}_3$  and submitted to CC (silica gel,  $15 \times 2.5$  cm,  $\text{CHCl}_3$  (1 l)). The product fractions containing both (*PR*)- and (*PS*)-isomers were evaporated, and their separation was achieved by prep. TLC (silica gel,  $\text{CH}_2\text{Cl}_2/\text{AcOEt}/\text{hexane}$  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  $40^\circ$ .

**4a** (*PR*):  $^{31}\text{P-NMR}$  ( $\text{CDCl}_3$ ): 69.84. Anal. calc. for  $\text{C}_{80}\text{H}_{98}\text{N}_{10}\text{O}_{14}\text{PSSi}_3 \cdot \text{H}_2\text{O}$  (1589.0): C 59.89, H 6.28, N 9.61; found: C 59.89, H 6.32, N 9.21.

**4b** (*PS*):  $^{31}\text{P-NMR}$  ( $\text{CDCl}_3$ ): 69.22. Anal. calc. for  $\text{C}_{80}\text{H}_{98}\text{N}_{10}\text{O}_{14}\text{PSSi}_3 \cdot \text{H}_2\text{O}$  (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*)- $\text{N}^6$ -Benzoyl-3'-O-[(*tert*-butyl)dimethylsilyl]-P-thioadenylyl-{2'-{O<sup>p</sup>-[2-(4-nitrophenyl)-ethyl]}→5'}- $\text{N}^6$ -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%  $\text{TsOH}$  in  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  4:1 (3.2 ml) at r.t. for 40 min. The mixture was taken up in  $\text{CHCl}_3$  (50 ml), washed with phosphate buffer pH 7.5 ( $2 \times 20$  ml), and evaporated to a foam. The residue was purified by CC (silica gel,  $10 \times 2.5$  cm, first  $\text{CHCl}_3$ , then  $\text{CHCl}_3/\text{MeOH}$  100:0.5 and 100:1, resp.). The product fractions were evaporated and dried at  $40^\circ/\text{h.v.}$ : 0.198 g (92%) of **5a** and 0.192 g (89%) of **5b**, resp.

**5a** (*PR*): TLC ( $\text{CH}_2\text{Cl}_2/\text{AcOEt}/\text{hexane}$  1:1:1):  $R_f$  0.27. Anal. calc. for  $\text{C}_{60}\text{H}_{82}\text{N}_{11}\text{O}_{13}\text{PSSi}_3 \cdot \text{H}_2\text{O}$  (1330.7): C 54.15, H 6.36, N 11.57; found: C 53.78, H 6.44, N 10.72.

**5b** (*PS*): TLC ( $\text{CH}_2\text{Cl}_2/\text{AcOEt}/\text{hexane}$  1:1:1):  $R_f$  0.30. Anal. calc. for  $\text{C}_{60}\text{H}_{82}\text{N}_{11}\text{O}_{13}\text{PSSi}_3$  (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  $\text{AcOH}$  in dry pyridine (4.5 ml) and then evaporated. The residue was taken up in 1M  $\text{Bu}_4\text{NF}$  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.  $\text{NH}_3$  soln. (20 ml) by stirring at r.t. for 48 h. After another evaporation, the residue was taken up in  $\text{H}_2\text{O}$  (50 ml) and washed with  $\text{CHCl}_3$  ( $2 \times 20$  ml) and the  $\text{H}_2\text{O}$  phase applied onto a *DEAE-Sephadex* column *A-25* ( $60 \times 1$  cm). Elution was performed with a linear gradient (0.001–0.25M) of  $(\text{Et}_3\text{NH})\text{HCO}_3$  buffer (pH 7.5). The product fractions were evaporated several times with  $\text{H}_2\text{O}$ . The  $\text{Et}_3\text{NH}^+$  salt was converted into its  $\text{NH}_4^+$  salt by paper chromatography using  $\text{i-PrOH}/\text{NH}_3/\text{H}_2\text{O}$  7:1:2 by  $\text{H}_2\text{O}$  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,  $\text{i-PrOH}/\text{conc. NH}_3$  soln./ $\text{H}_2\text{O}$  7:1:2):  $R_f$  (**6a**) 0.29;  $R_f$  (**6b**) 0.30.

5. (PR)- and (PS)-N<sup>6</sup>-Benzoyl-3'-O-[(tert-butyl)dimethylsilyl]-5'-O-(monomethoxytrityl)-P-thioadenylyl-{2'-{O<sup>P</sup>-[2-(4-nitrophenyl)ethyl]}→5'}-(PR)-N<sup>6</sup>-benzoyl-3'-O-[(tert-butyl)dimethylsilyl]-P-thioadenylyl-{2'-{O<sup>P</sup>-[2-(4-nitrophenyl)ethyl]}→5'}-N<sup>6</sup>-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-1H-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<sub>2</sub>Cl<sub>2</sub> (50 ml), the org. phase washed with sat. NaCl soln. (2 × 20 ml), dried (Na<sub>2</sub>SO<sub>4</sub>), 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<sub>3</sub>/MeOH 50:1) and the two diastereoisomers were then separated by prep. TLC (silica gel; 20 × 20 × 0.2 cm, CH<sub>2</sub>Cl<sub>2</sub>/AcOEt/hexane 1:1:0.5, 3 times). The product bands were eluted with CHCl<sub>3</sub>/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<sub>111</sub>H<sub>135</sub>N<sub>17</sub>O<sub>22</sub>P<sub>2</sub>S<sub>2</sub>Si<sub>4</sub>·H<sub>2</sub>O (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<sub>111</sub>H<sub>135</sub>N<sub>17</sub>O<sub>22</sub>P<sub>2</sub>S<sub>2</sub>Si<sub>4</sub>·H<sub>2</sub>O (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<sub>111</sub>H<sub>135</sub>N<sub>17</sub>O<sub>22</sub>P<sub>2</sub>S<sub>2</sub>Si<sub>4</sub> (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<sub>111</sub>H<sub>135</sub>N<sub>17</sub>O<sub>22</sub>P<sub>2</sub>S<sub>2</sub>Si<sub>4</sub>·H<sub>2</sub>O (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<sup>6</sup>-Benzoyl-3'-O-[(tert-butyl)dimethylsilyl]-P-thioadenylyl-{2'-{O<sup>P</sup>-[2-(4-nitrophenyl)ethyl]}→5'}-(PR)-N<sup>6</sup>-benzoyl-3'-O-[(tert-butyl)dimethylsilyl]-P-thioadenylyl-{2'-{O<sup>P</sup>-[2-(4-nitrophenyl)ethyl]}→5'}-N<sup>6</sup>-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<sub>2</sub>Cl<sub>2</sub>/MeOH 4:1 (1.5 ml) for 3 h. The soln. was taken up in CHCl<sub>3</sub> (50 ml) and washed with H<sub>2</sub>O (2 × 25 ml), the CHCl<sub>3</sub> phase dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated, and the crude product purified by prep. TLC (silica gel; 20 × 20 × 0.2 cm, CH<sub>2</sub>Cl<sub>2</sub>/AcOEt/hexane 1:1:0.5). The pure product was eluted from the plates with CHCl<sub>3</sub>/MeOH 1:1 giving in all cases 80% yields.

**8a** (PR,PR): Anal. calc. for C<sub>91</sub>H<sub>119</sub>N<sub>17</sub>O<sub>21</sub>P<sub>2</sub>S<sub>2</sub>Si<sub>4</sub>·H<sub>2</sub>O (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<sub>91</sub>H<sub>119</sub>N<sub>17</sub>O<sub>21</sub>P<sub>2</sub>S<sub>2</sub>Si<sub>4</sub> (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<sub>91</sub>H<sub>119</sub>N<sub>17</sub>O<sub>21</sub>P<sub>2</sub>S<sub>2</sub>Si<sub>4</sub> (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<sub>91</sub>H<sub>119</sub>N<sub>17</sub>O<sub>21</sub>P<sub>2</sub>S<sub>2</sub>Si<sub>4</sub> (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<sub>4</sub>NF in THF, and after stirring for 48 h, the solvent was evaporated and the residue treated with conc. NH<sub>3</sub> soln. (25 ml) for another 48 h. The solvent was evaporated and the residue dissolved in H<sub>2</sub>O (20 ml) and washed with CHCl<sub>3</sub> (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<sub>3</sub>NH)HCO<sub>3</sub> buffer (pH 7.5). The product fractions were evaporated and co-evaporated several times with H<sub>2</sub>O. The products were further purified by paper chromatography (i-PrOH/conc. NH<sub>3</sub> soln./H<sub>2</sub>O 6:1:3) to give, after lyophilisation, the fully deblocked trimers in 75–80% yields as colourless powders.

8. N<sup>6</sup>-Benzoyl-3'-O-[(tert-butyl)dimethylsilyl]-5'-O-{[2,5-dichlorophenoxy][2-(4-nitrophenyl)ethoxy]phosphoryl}-P-thioadenylyl-{2'-{O<sup>P</sup>-[2-(4-nitrophenyl)ethyl]}→5'}-N<sup>6</sup>-benzoyl-3'-O-[(tert-butyl)dimethylsilyl]-P-thioadenylyl-{2'-{O<sup>P</sup>-[2-(4-nitrophenyl)ethyl]}→5'}-N<sup>6</sup>-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 1H-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<sub>3</sub> (50 ml), the org. phase washed with H<sub>2</sub>O (2 × 20 ml), evaporated, and finally co-evaporated with toluene. The residue

was purified by prep. TLC (silica gel;  $20 \times 20 \times 0.2$  cm,  $\text{CH}_2\text{Cl}_2/\text{AcOEt}/\text{hexane}$  1:1:1). The product band was eluted with  $\text{CHCl}_3/\text{MeOH}$  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/ $\text{Et}_3\text{N}/\text{H}_2\text{O}$  (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 ( $2 \times 5$  ml), the product was purified by prep. TLC (silica gel;  $20 \times 20 \times 0.2$  cm,  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  95:5). The product band was eluted with  $\text{CHCl}_3/\text{MeOH}/\text{Et}_3\text{N}$  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  $\text{Bu}_4\text{NF}$  in THF (6 ml), and, after stirring at r.t. for 48 h and evaporation, debenzoylation was achieved by treatment with conc.  $\text{NH}_3$  soln. (25 ml) for 48 h at r.t. The mixture was evaporated, the residue taken up in  $\text{H}_2\text{O}$  (25 ml) and washed with  $\text{CHCl}_3$  ( $2 \times 10$  ml), and the aq. phase applied onto a DEAE-Sephadex A-25 column ( $60 \times 1$  cm) for elution with a linear gradient (0.001–1M) of  $(\text{Et}_3\text{NH})\text{HCO}_3$  buffer (pH 7.5). The product fractions were evaporated and co-evaporated several times with  $\text{H}_2\text{O}$ . Further purification by paper chromatography (i-PrOH/conc.  $\text{NH}_3$  soln./ $\text{H}_2\text{O}$  55:10:35) gave, after lyophilisation, colourless powders of the trimer 5'-monophosphates in 68–74% yield.

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