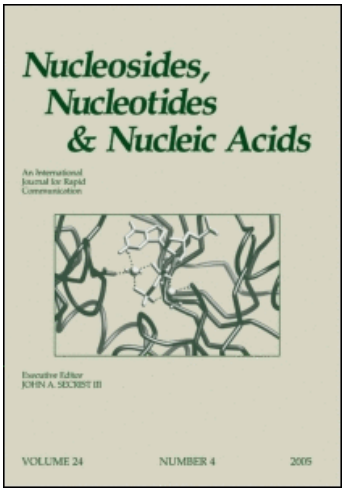

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Stereoselective Synthesis of Ribonucleoside 3',5'-Cyclic Methyl(phenyl)phosphonates and Phosphonothioates

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STEREOSELECTIVE SYNTHESIS OF RIBONUCLEOSIDE 3',5'-CYCLIC METHYL(PHENYL)PHOSPHONATES AND PHOSPHONOTHIOATES

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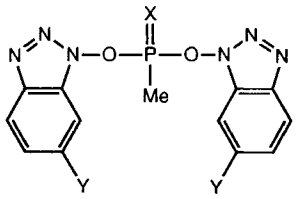
ABSTRACT

Monophosphonylation of 2'-protected ribonucleosides (*i.e.*. 2'-*O*-THP-uridine and 2'-*O*-THP-N^6-levulinoyl-adenosine) with the bifunctional reagents *bis*[(6-trifluoromethyl)benzotriazol-1-yl] methyl(phenyl)phosphonates or the analogous phosphonothioates, and subsequent addition of *N*-methylimidazole, gave the chirally pure 3',5'-cyclic methyl(phenyl)phosphonate or phosphonothioate derivatives, respectively. Deblocking of the fully protected compounds yielded, as evidenced by X-ray analysis, the corresponding pure Sp-diastereoisomers.

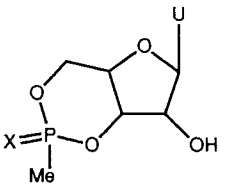
INTRODUCTION

It is well recognized that low molecular weight nucleic acids regulate a plethora of biological processes.[1] For example, adenosine 5'-triphosphate (ATP) is essential for the energy household of nearly all cells, and the tetra- or pentaphosphate derivatives of riboguanosine (*i.e.* ppGpp and pppGpp: so called magic spots) play an essential role[2] in the stringent control mechanism of RNA-synthesis in *E-coli* cells. Recently, it was also found[3,4] that 3',5'-cyclic diriboguanylic acid (cGpGp) functions as an activator of the enzyme cellulose synthase in the Gram-negative bacterium *Acetobacter xylinum.* Apart from this, it is well established that adenosine 3',5'-cyclic phosphate (cAMP) is a key regulator of metabolism, function and growth of many cell types.[5] For instance cell aggregation in the amoeba *Dictyostelium discoideum* is mediated by chemotaxis to cAMP.[6] Thus far, the chemotaxic response of several cAMP analogues has been studied.[7] The outcome of this study revealed *inter alia* that the Sp-diastereoisomer of adenosine 3',5'-cyclic phosphorothioate (Sp-cAMPS) evoked a high chemotaxic response in *D.*

discoideum cells. In earlier studies, directed towards the introduction of modified internucleotidic phosphodiester linkages (*e.g.* methylphosphonate[8] and methylphosphonothioate[9] bonds), we established that monophosphonylation of a properly 2'-protected uridine derivative with the bifunctional reagents **1** and **2** followed by cyclisation resulted, as evidenced by ^{1}H- and ^{31}P-NMR spectroscopy, in the exclusive formation of the chirally pure methylphosphonate **3** and methylphosphonothioate **4**, respectively.



1 X = O, Y = H (BT)
2 X = S, Y = CF_3 (BTFM)



3 X = O
4 X = S

We report herein that the two-step cyclisation process can also be executed with the analogous phenyl derivatives (*i.e.*, **5** and **7**) of the reagents **1** and **2**. Further, the two-step cyclisation process afforded in each case the diastereoisomerically pure (*i.e.* Sp-configuration) methyl(phenyl)-phosphonate or corresponding phosphonothioate analogues of cUMP and cAMP.

RESULTS AND DISCUSSION

In order to consolidate our earlier reported synthesis of the cyclic uridine derivatives **3** and **4**, we phosphonylated (see Scheme 1) in the first place 2'-*O*-tetrahydropyranyl-uridine [**8**, higher running diastereoisomer (hrd)] with a slight excess of the *in situ* prepared reagent *bis*[6-(trifluoromethyl)benzotriazol-1-yl] methylphosphonothioate (**2**) in dioxane. Monitoring of the phosphonylation step by ^{31}P-NMR revealed the rapid appearance of two resonances at 114.42 and 110.94 ppm, which we assigned to the individual diastereoisomers of the putative intermediate **11**. After completion of the reaction (5 min. at 20°C), an excess of *N*-methylimidazole was added to intermediate **11**. Interestingly, ^{31}P-NMR analysis of the cyclisation step revealed that both diastereoisomers of **11** were converted at the same rate into a product having a δp-value of 102.11 ppm. Work up, after 1 h at 20°C, gave the fully protected and diastereoisomerically pure cyclic product **15** in an excellent yield (see Table 1). However, the two-step cyclisation of **8** (hrd) with reagent **6**, similarly activated as **2** with the 6-trifluoromethylbenzotriazol-1-yl (BTFM) group, afforded the chirally pure oxygen analogue **25** in a much lower yield (see Table 1).

Scheme 1

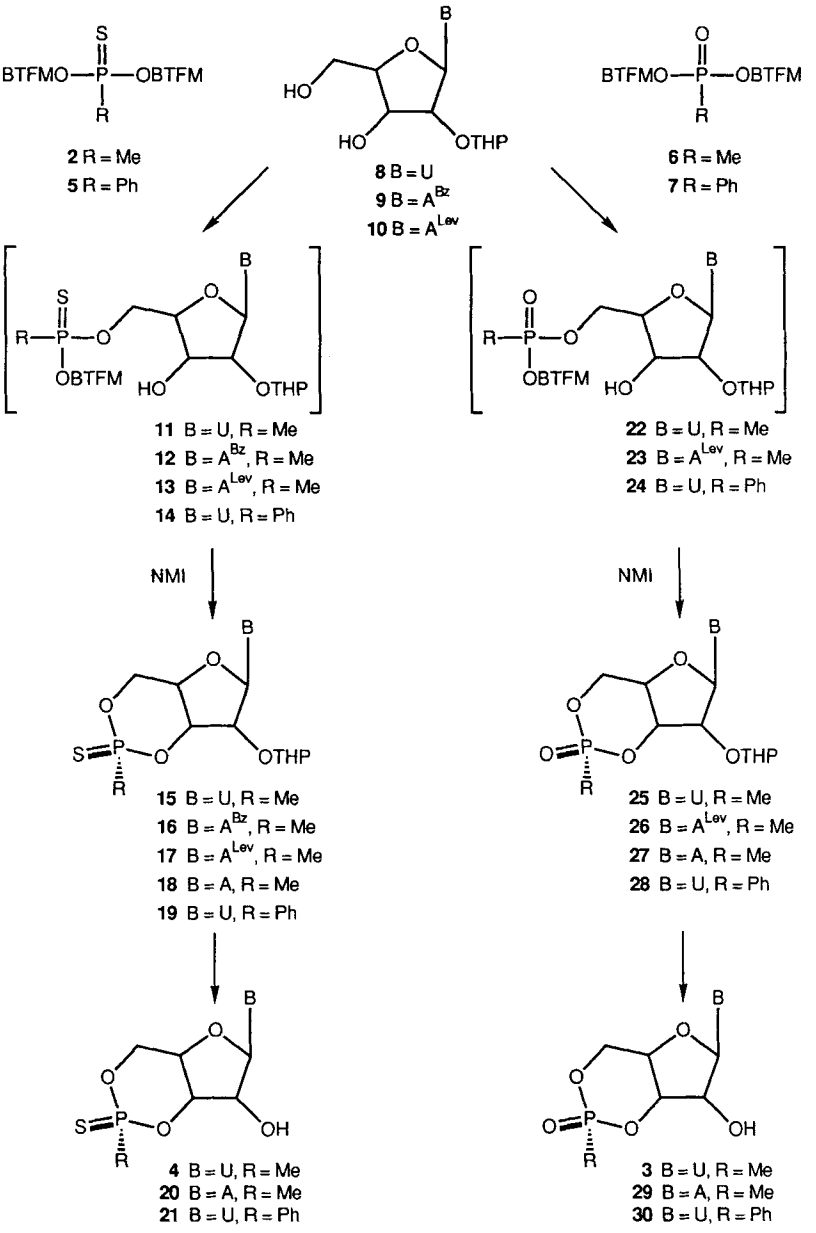


TABLE 1. Relevant data of fully and partially protected 3',5'-cyclic phosphon(othio)ates **15-19** and **25-28**.

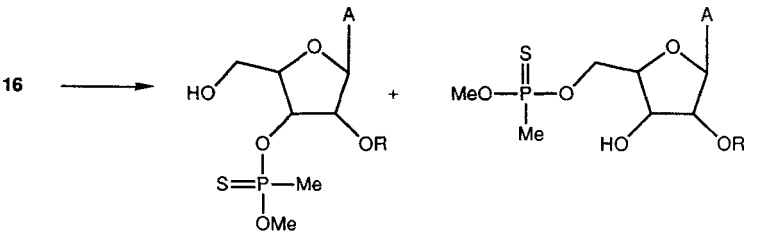
Compound	Yield (%)	R_f-values[a]	^{31}P NMR[b]	^{1}H NMR[c] (H-1')[d]	^{13}C NMR[c] (C-1')
15	81	0.63	102.3	5.86	91.2
16	88	0.61	99.9	6.15	90.2
17	68	0.60	101.8	6.21	90.3
18	90	0.53	102.0	6.10	89.9
19	60	0.66	90.6	5.92	91.3
25	39	0.34	34.2	5.80	91.4
26	46	0.54	32.7	6.28	89.6
27	89	0.37	32.3	6.09	89.9
28	71	0.47	19.3	5.97	91.2

[a] Eluens: CH_2Cl_2/CH_3OH (92/8, v/v). [b] ^{31}P NMR chemical shifts (δ-values) in ppm relative to 85% H_3PO_4. Samples were measured in CH_2Cl_2 and D_2O was used as external reference. [c] Chemical shifts (δ-values) in ppm relative to tetramethylsilane (TMS). Samples were measured in $CDCl_3$. [d] The pattern of the H-1' proton was in each case a singlet.

On the other hand, ^{31}P-NMR analysis of the two-step cyclisation process showed a similar sequence of events as observed for the conversion of **8** into **15**. Thus, the two phosphorus resonances at 40.16 and 39.65 ppm of intermediate **22** disappeared simultaneously to give one resonance at 31.88 ppm. Cleavage of the tetrahydropyranyl (THP) group from **15** by acidic hydrolysis[10] afforded **4**, the ^{1}H-, ^{13}C- and ^{31}P-NMR data of which (see Table 2) were in complete accord with the presence of one diastereoisomer.

In a similar fashion, the optically pure N^6-benzoyl-adenosine derivative **16** was prepared in a good yield (see Table 1) by phosphonothiolation of **9** (hrd) with **2** followed by cyclisation of intermediate **12**. Unfortunately, ammonolysis (NH_3/MeOH) of the N^6-benzoyl group from **16** did not give the debenzoylated derivative **16** (B=A) but, as will be demonstrated below, the products **31** and **32** resulting from a nucleophilic attack of methoxide ion on the neutral phosphorus atom and concomitant non-selective ring opening (see Scheme 2). Thus work up and

Scheme 2



31 R = THP
33 R = H

32 R = THP
34 R = H

purification of the reaction mixture gave, after removal of the THP group, two products with different R_f-values. ^{1}H-NMR spectroscopy of the individual products, isolated after column chromatography, revealed *inter alia* a doublet for each of the H-1' protons: thus indicating the absence (see footnote *d* in Table 2) of a 3',5'-cyclic phosphonate function. Further, extensive ^{1}H-, ^{13}C- and ^{31}P-NMR spectroscopic analysis indicated that the structures of the products, resulting from the high-yielding two-step deblocking of **16**, were in complete accordance with those of the chirally pure positional isomers **33** and **34**.

In order to prevent the above-referenced side reaction, we prepared the adenosine derivative **10**, the N^6-levulinoyl group[13] of which can be removed under extremely mild conditions with hydrazine in pyridine-acetic acid.[11] The preparation of **10** could be realized by condensation of 2'-*O*-tetrahydropyranyl-3',5'-*O*,*O*-(tetraisopropyldisiloxane-1,3-diyl)-adenosine[12] with levulinic acid in the presence of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline[13], and subsequent removal of the 3',5'-tetraisopropyldisilyl group with fluoride ions. Phosphonothiolation of **10** (Ird) with **2** followed by *N*-methylimidazole-assisted cyclisation of intermediate **13** afforded **17** (one diastereoisomer). In a similar way, the analogous methyl-phosphonate derivative **26** was obtained as a pure diastereoisomer by treating **10** with reagent **6**. Yields and other relevant data for compounds **17** and **26** are summarized in Table 1. Hydrazinolysis of **17** and **25** followed by acidic hydrolysis of the obtained derivatives **18** and **27** as described above afforded (see Table 2) the corresponding diastereoisomerically pure cyclic derivatives **20** and **29**.

We further demonstrated that the two-step transformation of **8** (hrd) via the intermediates **14** (δp 98.37 and 97.76) and **24** (δp 26.02 and 25.54) into the

TABLE 2. Relevant data of fully deprotected ribonucleoside 3',5'-cyclic phosphon(othio)ates **3**, **4**, **20**, **21**, **29** and **30**.

Compound	Yield (%)	R_f-values[a] A B	^{31}P NMR[b]	^{1}H NMR[c] (H-1')[d]	^{13}C NMR[c] (C-1')
4	83	0.35, 0.53	103.1	5.75[e]	96.2[e]
20	78	0.16, 0.25	103.4	5.77[f]	92.3[f]
21	73	0.41, 0.59	90.6	5.70[g]	96.5[g]
3	82	0.18, 0.32	37.5	5.78[e]	95.4[e]
29	77	0.14, 0.20	35.2	6.06[f]	91.6[f]
30	69	0.30, 0.45	20.7	5.67[g]	95.2[g]

[a] Eluents: A: CH_2Cl_2/CH_3OH (92/8, v/v) and B: CH_2Cl_2/CH_3OH (85/15, v/v). [b] ^{31}P NMR chemical shifts (δ-values) in ppm relative to 85% H_3PO_4. Samples were measured in CH_2Cl_2/CH_3OH or in CH_3OH in case of compound **21** and D_2O was used as external reference. [c] Chemical shifts (δ-values) in ppm relative to tetramethylsilane (TMS). [d] The pattern of the H-1' proton was in each case a singlet. [e] Samples were measured in D_2O. [f] Samples were measured in $CDCl_3/CD_3OD$. [g] Samples were measured in $(CD_3)_2SO/CD_3OD$.

chirally pure phenyl derivatives **19** and **28** could be accomplished using the easily accessible phenylphosphonylating reagents **5** and **7**, respectively. In this respect it is interesting to note that the yield of the phenylphosphonate **28** was much higher than of the corresponding methylphosphonates **25-26** (see Table 1). Removal of the THP groups from **19** and **28** gave the deprotected 3',5'-cyclic phenylphosphonates **21** and **30**, respectively (see Table 2).

Finally, the Sp-configuration of the non-charged cyclic phosphate analogues of cUMP (*i.e.*, **3**, **4**, **21** and **30**) and cAMP (*i.e.*, **20** and **29**) was unambiguously ascertained[14] by X-ray diffraction analysis (see Figure 1).

The diastereoselective outcome of the two-step cyclisation process[15] may be rationalized as follows. The first step, which involves treatment of the partially protected ribonucleosides **8-10** with the bifunctional reagents **2,5-7**, leads to the formation of the corresponding intermediates **11-14** and **22-24** consisting in each case of a nearly equal amount of the corresponding Sp- and Rp-diastereoisomers. Addition, in the second step, of *N*-methylimidazole will convert the initially released 1-hydroxy(6-trifluoromethyl)benzotriazole into the benzotriazol-1-yl-oxide ion. The

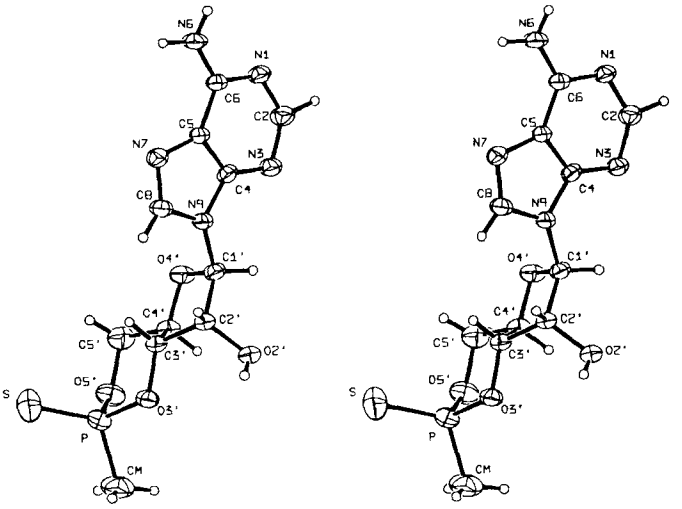


FIG. 1. An ORTEP stereoview of adenosine 3',5'-cyclic methylphosphonothioate **20**

high tendency of the latter ion towards nucleophilic attack on the pentacovalent phosphorus atom in the open-form intermediates **11-14** and **22-24** may ensue a rapid equilibration between the individual Rp- and Sp-diastereoisomers.

In addition, *N*-methylimidazole will enhance, presumably via intermediate hydrogen bonding, the nucleophilicity of the 3'-OH in the open-form intermediates. The activated hydroxyl group now reacts selectively with the Rp- diastereoisomer to give the Sp-cyclic products **15-19** and **25-28** having the methyl(phenyl) substituents in a thermodynamically more stable equatorial position. The Rp-diastereoisomer consumed in the cyclisation step will then be replenished rapidly by the (6-trifluoromethyl)benzotriazol-1-yl-oxide ion-mediated inversion of the Sp-diastereoisomer.

Finally the Sp-diastereoisomers of the cAMP analogue **20** and **29** did not evoke any detectable chemotaxicity in *D. discoideum* cells.

EXPERIMENTAL

Materials and methods. Dioxane, *N*-methylimidazole, pyridine and tetrahydrofuran (THF) were dried by refluxing with CaH_2 for 16 h and then distilled. Dioxane and THF were redistilled from $LiAlH_4$ (5 g/L) and stored on molecular sieves 5Å. Pyridine was redistilled from *p*-toluenesulfonyl chloride (60 g/L) and KOH (25 g/L) and stored on molecular sieves 4Å. *N,N*-Dimethylformamide was dried by stirring overnight at room temperature with CaH_2

(5 g/L) and distilled under reduced pressure. Methylphosphonic dichloride (Janssen), methylthiophosphonic dichloride (Fluka), phenylphosphonic dichloride (Janssen), phenylthiophosphonic dichloride (Janssen), levulinic acid (LevOH, Aldrich) and 2,3-dihydropyran (DHP, Janssen) were distilled before use. *N*-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, Aldrich), potassium fluoride (KF, Baker), tetraethylammonium bromide (Et_4NBr, Fluka), mesitylenesulfonic acid (MSA, Aldrich), hydrazine monohydrate (Janssen) and pentane-2,4-dione (Aldrich) were used without further purification. 1-Hydroxy-6-trifluoromethyl-benzotriazole was prepared according to the procedure of König and Geiger[16] and dried *in vacuo* (P_2O_5) for 72 h at 50°C before use. 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPDSiCl_2$) and partially protected nucleosides **8** and **9** were prepared as described previously.[12] Triethylammonium bicarbonate (TEAB, 2M) buffer was prepared by passing a stream of CO_2-gas through a cooled (ice-water bath) mixture of triethylamine (825 mL) in deionized water (2175 mL) until a neutral solution (pH 7.0-7.5) was obtained. Schleicher and Schüll DC Fertigfolien F1500 LS254 were used for TLC in CH_2Cl_2/CH_3OH (92/8, v/v), unless otherwise stated. The phosphorylation reactions were quenched on TLC-sheets with a mixture of pyridine/water (1/1, v/v). Short column chromatography was performed on Kieselgel 60 (230-400 mesh ASTM) suspended in CH_2Cl_2 or on Sephadex LH 20 suspended in CH_2Cl_2/CH_3OH (2/1, v/v). ^1H-, ^{13}C- and ^{31}P NMR spectra were measured at respectively 200 MHz, 50.1 MHz and 80.7 MHz using a JEOL JNM-FX 200 Fourier Transform NMR Spectrometer, equipped with a PG 200 computer and operating in the Fourier transform mode. Chemical shifts are given in ppm (δ) relative to tetramethylsilane for ^1H- and ^{13}C NMR and relative to 85% H_3PO_4 for ^{31}P NMR.

***Bis*[1-(6-trifluoromethyl)benzotriazol-1-yl]methylphosphonothioate (2).**[9] A solution of methylphosphonothioic dichloride (1.08 g, 0.77 mL, 7.25 mmol, ^{31}P NMR [D_2O, external lock]: δ 83.7 ppm) in anhydrous dioxane (7.00 mL) was added dropwise to a stirred solution of dry 1-hydroxy-6-trifluoromethylbenzotriazole (3.01 g, 14.8 mmol) and anhydrous pyridine (1.20 mL, 15.0 mmol) in anhydrous dioxane (30.0 mL) at room temperature. After stirring for 2 h at 20°C, the pyridinium-HCl salt was removed by filtration under anhydrous conditions. The 0.20 M stock solution of **2** thus obtained could be stored for several weeks at -20°C. ^{31}P NMR (D_2O, external lock): δ 122.2 (s).

***Bis*[1-(6-trifluoromethyl)benzotriazol-1-yl]phenylphosphonothioate (5).** Reaction of phenylphosphonothioic dichloride (1.53 g, 1.13 mL, 7.25 mmol, ^{31}P NMR [D_2O, external lock]: δ 76.0 ppm) with 1-hydroxy-6-trifluoromethyl-benzotriazole (3.01 g, 14.8 mmol) in a similar way as described for the preparation of **2** afforded phosphorylating agent **5**. ^{31}P NMR (D_2O, external lock): δ 106.7 (s).

***Bis*[1-(6-trifluoromethyl)benzotriazol-1-yl]methylphosphonate (6).** Reaction of methylphosphonic dichloride (0.96 g, 7.25 mmol, ^{31}P NMR [D_2O, external lock]: δ 43.94 ppm) with 1-hydroxy-6-trifluoromethyl-benzotriazole (3.01 g, 14.8 mmol) in a similar way as described for the preparation of **2** afforded phosphorylating agent **6**. ^{31}P NMR (D_2O, external lock): δ 46.6 (s).

***Bis*[1-(6-trifluoromethyl)benzotriazol-1-yl]phenylphosphonate (7).** Reaction of phenylphosphonic dichloride (1.41 g, 1.03 mL, 7.25 mmol, ^{31}P NMR [D_2O, external lock]: δ 35.8 ppm) with 1-hydroxy-6-trifluoromethyl-benzotriazole (3.01 g, 14.8 mmol) in a similar way as described for the preparation of **2** afforded phosphorylating agent **7**. ^{31}P NMR (D_2O, external lock): δ 40.6 (s).

2'-*O*-Tetrahydropyranyl-*N*-6-levulinoyl-adenosine (10). To a stirred solution of 2'-*O*-tetrahydropyranyl-3',5'-*O*,*O*-(tetraisopropyldisiloxane-1,3-diyl)-adenosine[12] (5.95 g, 10.0 mmol) in THF (200 mL) were added levulinic acid (11.6 g, 100 mmol) and EEDQ (29.7 g, 120 mmol). After stirring for 6 h at 80°C, TLC analysis indicated the complete formation of a new product [R_f 0.81 (CH_2Cl_2/CH_3OH, 96/4, v/v)]. The reaction mixture was concentrated and the residual syrup was dissolved in ether (200 mL) and washed with cold aqueous HCl (0.01 M, 50 mL) and water (50 mL). The organic layer was concentrated to near dryness and dissolved in acetonitrile (100 mL). To the resulting solution was added an aqueous solution of KF (5.0 M, 10 mL) and Et_4NBr (10 g), and the mixture was stirred for 1 h at 50°C. TLC analysis indicated complete removal of the TIPS-group. The reaction mixture was concentrated under reduced pressure, the residue dissolved in CH_2Cl_2 (200 mL) and washed with an aqueous solution of $NaHCO_3$ (5% w/v, 50 mL) and H_2O (50 mL). The organic layer was dried ($MgSO_4$), concentrated to a small volume (10 mL) and purified by short column chromatography, applying a 0 to 6% gradient of CH_3OH. The individual diastereoisomers of **10** were separated, collected and concentrated to a glass. Yield of the high running diastereoisomer **10** was 1.56 g (3.47 mmol, 35%); R_f 0.52; ^1H NMR ($CDCl_3$): δ 1.36-1.71 (m, 6H, THP), 3.21 (s, 3H, CH_3 [lev]), 3.28 (m, 4H, 2 x CH_2 [lev]), 3.80 (m, 1H, THP), 3.98 (m, 1H, THP), 4.38-4.45 (m, 2H, H-5' and H-5''), 4.50-4.89 (m, 3H, H-2', H-3' and H-4'), 6.02 (d, $J_{1',2'}$ = 7.3 Hz, 1H, H-1'), 8.19 (s, 1H, H-2), 8.68 (s, 1H, H-8); ^{13}C NMR ($CDCl_3$): δ 20.3 (s, THP), 24.2 (s, THP), 29.3 (s, CH_3 [lev]), 30.1 (s, THP), 31.1 (s, $\underline{C}H_2$-C(=O)-CH_3 [lev]), 37.0 (s, $\underline{C}H_2$-C(=O)-NH [lev]), 62.0 (s, THP), 64.7 (s, C-5'), 71.0 (s, C-3'), 81.0 (s, C-2'), 86.6 (s, C-4'), 88.3 (s, C-1'), 100.9 (s, THP), 122.1 (s, C-5), 142.5 (s, C-8), 149.1 (s, C-4), 151.0 (s, C-2), 151.2 (s, C-6), 171.3 (s, NH-C=O [lev]), 206.7 (s, H_3C-$\underline{C}$=O [lev]). Yield of the low running diastereoisomer **10** was 1.89 g (4.20 mmol, 42%); R_f 0.44; ^1H NMR ($CDCl_3$): δ 1.30-1.68 (m, 6H, THP), 3.14 (s, 3H, CH_3 [lev]), 3.28 (m, 4H, CH_2 [lev]), 3.77 (m, 1H, THP), 3.92 (m, 1H, THP), 4.39-4.45 (m, 2H, H-5' and H-5''), 4.46-4.80 (m, 3H, H-2', H-3' and H-4'), 5.94 (d, $J_{1',2'}$ = 7.0 Hz, 1H, H-1'), 8.11 (s, 1H, H-2), 8.67 (s, 1H, H-8); ^{13}C NMR ($CDCl_3$): δ 20.3 (s, THP), 24.3 (s, THP), 29.1 (s, CH_3 [lev]), 30.1 (s, THP), 31.1 (s, $\underline{C}H_2$-C(=O)CH_3 [lev]), 37.1 (s, $\underline{C}H_2$-C(=O)NH [lev]), 62.0 (s, THP), 65.0 (s, C-5'), 71.0 (s, C-3'), 80.1 (s, C-2'), 86.4 (s, C-4'), 88.0 (s, C-1'), 100.7 (s, THP), 119.7 (s, C-5), 140.5 (s, C-8), 149.0 (s, C-4), 152.2 (s, C-2), 153.9 (s, C-6), 171.9 (s, NH-C=O [lev]), 206.7 (s, H_3C-$\underline{C}$=O [lev]).

General procedure for the synthesis of ribonucleoside 3',5'-cyclic phosphonothioates 15, 16, 17 and 19 via intermediates 11-14. A solution of phosphorylating agent **2** or **5** (0.20 M, 5.5 mL, 1.1 mmol) in dioxane was added to the individual ribonucleosides **8-10** (1.0 mmol) which had been dried by repeated coevaporation with anhydrous pyridine (3 x 20 mL). The reaction mixture was stirred for 5 min (in case of **2**) and 30 min (in case of **5**) at 20°C. *N*-Methylimidazole (0.40 mL, 5.0 mmol) was added, and the reaction mixture was stirred for 1 h (in case of **2**) and 4 h (in case of **5**) at 20°C. A few drops of TEAB buffer (1 M) was added, the reaction mixture diluted with CH_2Cl_2 (50 mL) and washed twice with TEAB buffer (1 M, 50 mL; 0.1 M, 50 mL). The organic layer was dried ($MgSO_4$), concentrated to a small volume, coevaporated with toluene (2 x 50 mL) and finally evaporated with CH_2Cl_2 (1 x 50 mL) to a colourless foam. Crude products **15**, **16**, **17** and **19** were purified by short column chromatography using a gradient of CH_3OH in CH_2Cl_2.

2'-*O*-Tetrahydropyranyl-uridine 3',5'-cyclic methylphosphonothioate (15). 3',5'-Cyclic phosphonothioate **15** was prepared from **8** (high running diastereoisomer, 328 mg, 1.0 mmol) and **2** (0.2 M, 5.5 mL, 1.1 mmol) as described above. Compound **15** was purified by

short column chromatography applying a 0 to 4% gradient of CH_3OH. Yield: 326 mg (0.81 mmol, 81%); R_f 0.63; ^1H NMR ($CDCl_3$): δ 1.50-1.82 (m, 6H, THP), 2.08 (d, J_{HP} = 15.8 Hz, 3H, P-CH_3), 3.56 (m, 1H, THP), 3.86 (m, 1H, THP), 4.20-4.74 (m, 5H, H-2', H-3', H-4', H-5' and H-5''), 5.13 (m, 1H, THP), 5.82 (d, J = 8.2 Hz, 1H, H-5), 5.86 (s, 1H, H-1'), 7.31 (d, J = 8.2 Hz, 1H, H-6); ^{13}C NMR ($CDCl_3$): δ 18.3 (s, THP), 20.7 (d, J_{CP} = 109.9 Hz, P-CH_3), 25.0 (s, THP), 29.9 (s, THP), 61.4 (s, THP), 66.4 (d, J_{CP} = 7.3 Hz, C-5'), 71.7 (d, J_{CP} = 4.4 Hz, C-2'), 72.9 (d, J_{CP} = 4.4 Hz, C-4'), 74.9 (d, J_{CP} = 7.3 Hz, C-3'), 91.2 (s, C-1'), 96.2 (s, THP), 102.9 (s, C-5), 138.5 (s, C-6), 149.8 (s, C-2), 163.2 (s, C-4); ^{31}P NMR (CH_2Cl_2): δ 102.3 (s).

2'-*O*-Tetrahydropyranyl-*N*-6-benzoyl-adenosine 3',5'-cyclic methylphosphonothioate (16). 3',5'-Cyclic phosphonothioate **16** was prepared from **9** (low running diastereoisomer, 455 mg, 1.0 mmol) and **2** (0.20 M, 5.5 mL, 1.1 mmol) as described above. Compound **16** was purified by short column chromatography applying a 0 to 4% gradient of CH_3OH. Yield: 0.47 g (0.88 mmol, 88%); R_f 0.61; ^1H NMR ($CDCl_3$): δ 1.51-1.89 (m, 6H, THP), 2.08 (d, J_{HP} = 15.9 Hz, 3H, P-CH_3), 3.50 (m, 1H, THP), 3.89 (m, 1H, THP), 4.29-4.59 (m, 2H, H-5' and H-5''), 4.72-4.87 (m, 3H, H-2', H-3' and H-4'), 5.13 (m, 1H, THP), 5.25 (m, 1H, H-3'), 6.15 (s, 1H, H-1'), 7.18 (m, 2H, arom. H's [Bz]), 7.51 (m, 2H, arom. H's [Bz]), 8.01 (d, J = 7.4 Hz, 1H, arom. H [Bz]), 8.07 (s, 1H, H-2), 8.80 (s, 1H, H-8); ^{13}C NMR ($CDCl_3$): δ 18.7 (s, THP), 21.0 (d, J_{CP} = 108.4 Hz, P-CH_3), 25.1 (s, THP), 30.1 (s, THP), 62.0 (s, THP), 66.8 (d, J_{CP} = 7.4 Hz, C-5'), 72.3 (d, J_{CP} = 5.9 Hz, C-2'), 73.1 (d, J_{CP} = 4.4 Hz, C-4'), 74.8 (d, J_{CP} = 7.9 Hz, C-3'), 90.2 (s, C-1'), 96.6 (s, THP), 123.5 (s, C-5), 127.9, 128.2, 128.8, 129.0, 132.8, 133.4 (6 x s, C-arom. [Bz]), 139.1 (s, C-8), 149.7 (s, C-4), 151.0 (s, C-6), 153.0 (s, C-2), 164.7 (s, C=O [Bz]); ^{31}P NMR (CH_2Cl_2): δ 99.9 (s).

2'-*O*-Tetrahydropyranyl-*N*-6-levulinoyl-adenosine 3',5'-cyclic methylphosphonothioate (17). 3',5'-Cyclic phosphonothioate **17** was prepared from **10** (low running diastereoisomer, 449 mg, 1.0 mmol) and **2** (0.2M, 5.5 mL, 1.1 mmol) in a similar way as described above. Compound **17** was purified by short column chromatography applying a 0 to 4% gradient of CH_3OH. Yield: 0.36 g (0.68 mmol, 68%); R_f 0.60; ^1H NMR ($CDCl_3$): δ 1.60-1.91 (m, 6H, THP), 2.11 (d, J_{HP} = 15.8 Hz, 3H, P-CH_3), 2.27 (s, 3H, CH_3 [lev]), 2.94 (t, J = 6.7 Hz, 2H, $C\underline{H}_2$-C(=O)CH_3 [lev]), 3.24 (t, J = 6.7 Hz, 2H, $C\underline{H}_2$-C(=O)NH [lev]), 3.47 (m, 1H, THP), 3.80 (m, 1H, THP), 4.12-4.57 (m, 2H, H-5' and H-5''), 4.64-4.91 (m, 3H, H-2', H-4' and THP), 5.59 (m, 1H, H-3'), 6.21 (s, 1H, H-1'), 8.28 (s, 1H, H-2), 8.74 (s, 1H, H-8); ^{13}C NMR ($CDCl_3$): δ 19.3 (s, THP), 20.9 (d, J_{CP} = 109.9 Hz, P-CH_3), 24.7 (s, THP), 29.7 (s, CH_3 [lev]), 30.0 (s, THP), 31.6 (s, $\underline{C}H_2$-C(=O)CH_3 [lev]), 37.4 (s, $\underline{C}H_2$-C(=O)NH [lev]), 63.0 (s, THP), 66.6 (d, J_{CP} = 7.3 Hz, C-5'), 71.9 (d, J_{CP} = 4.4 Hz, C-2'), 73.8 (d, J_{CP} = 4.4 Hz, C-4'), 76.3 (d, J_{CP} = 7.3 Hz, C-3'), 90.3 (s, C-1'), 99.8 (s, THP), 122.0 (s, C-5), 138.8 (s, C-8), 149.2 (s, C-4), 150.4 (s, C-6), 151.8 (s, C-2), 172.3 (s, NH-C=O [lev]), 207.0 (s, H_3C-$\underline{C}$=O [lev]); ^{31}P NMR (CH_2Cl_2): δ 101.8 (s).

2'-*O*-Tetrahydropyranyl-adenosine 3',5'-cyclic methylphosphonothioate (18). Hydrazine monohydrate (0.25 mL, 5.0 mmol) and glacial acetic acid (2.0 mL) in pyridine (5.0 mL)[11] were added to a cooled (0°C) solution of **17** (0.26 g, 0.50 mmol) in pyridine (2 mL). After stirring for 15-30 min at 20°C, the reaction mixture was cooled to 0°C and pentane-2,4-dione (1.0 mL, 10 mmol) was added. After stirring for 3 min, the mixture was taken up in CH_2Cl_2 (50 mL) and washed twice with TEAB buffer (1 M, 100 mL; 0.1 M, 100 mL). The organic layer was dried ($MgSO_4$), concentrated to a small volume (3 mL) and triturated with petroleum-ether (40-60°C, 75 mL). After filtration of the precipitate, crude **18** thus obtained

was purified by short column chromatography, applying a 0 to 6% gradient of CH_3OH. Yield: 189 mg (0.45 mmol, 90%); R_f 0.53; ^1H NMR ($CDCl_3$): δ 1.65-2.01 (m, 6H, THP), 2.09 (d, J_{HP} = 16.1 Hz, 3H, P-CH_3), 3.53 (m, 1H, THP), 3.89 (m, 1H, THP), 4.26-4.52 (m, 2H, H-5' and H-5''), 4.54-4.88 (m, 2H, H-2' and H-4'), 5.24 (m, 2H, H-3' and THP), 6.10 (s, 1H, H-1'), 6.23 (s, 2H, NH_2), 7.90 (s, 1H, H-2), 8.35 (s, 1H, H-8); ^{13}C NMR ($CDCl_3$): δ 18.5 (s, THP), 20.9 (d, J_{CP} = 109.9 Hz, P-CH_3), 25.1 (s, THP), 30.0 (s, THP), 61.7 (s, THP), 66.9 (d, J_{CP} = 7.4 Hz, C-5'), 72.0 (d, J_{CP} = 4.4 Hz, C-2'), 73.1 (d, J_{CP} = 4.4 Hz, C-4'), 74.8 (d, J_{CP} = 7.9 Hz, C-3'), 89.9 (s, C-1'), 96.3 (s, THP), 119.7 (s, C-5), 138.1 (s, C-8), 148.9 (s, C-4), 153.3 (s, C-2), 155.6 (s, C-6); ^{31}P NMR (CH_2Cl_2): δ 102.0 (s).

2'-*O*-Tetrahydropyranyl-uridine 3',5'-cyclic phenylphosphonothioate (19). 3',5'-Cyclic phosphonate **19** was prepared from **8** (high running diastereoisomer, 328 mg, 1.0 mmol) and **5** (0.20 M, 5.5 mL, 1.1 mmol) in a similar way as described above. Compound **19** was purified by short column chromatography applying a 0 to 4% gradient of CH_3OH. Yield: 564 mg (1.21 mmol, 60%); R_f 0.66 ; ^1H NMR ($CDCl_3$): δ 1.26-1.95 (m, 6H, THP), 3.41 (m, 1H, THP), 3.73 (m, 1H, THP), 4.37-4.84 (m, 5H, H-2', H-3', H-4', H-5' and H-5''), 5.19 (m, 1H, THP), 5.84 (d, $J_{5,6}$ = 8.4 Hz, 1H, H-5), 5.92 (s, 1H, H-1'), 7.21 (m, 1H, arom. H [phenyl]), 7.34 (d, $J_{5,6}$ = 8.5 Hz, 1H, H-6), 7.41-7.65 (m, 2H, arom. H's [phenyl]), 7.93-8.11 (m, 2H, arom. H's [phenyl]); ^{13}C NMR ($CDCl_3$): δ 17.8 (s, THP), 24.8 (s, THP), 29.7 (s, THP), 60.6 (s, THP), 67.3 (d, J_{CP} = 7.3 Hz, C-5'), 71.9 (d, J_{CP} = 4.4 Hz, C-2'), 73.7 (d, J_{CP} = 4.4 Hz, C-4'), 74.6 (d, J_{CP} = 7.3 Hz, C-3'), 91.3 (s, C-1'), 95.6 (s, THP), 102.7 (s, C-5), 127.7, 128.0, 128.3, 131.4, 132.6, 133.4 (C arom. [phenyl]), 138.6 (s, C-6), 149.8 (s, C-2), 163.2 (s, C-4); ^{31}P NMR (CH_2Cl_2): δ 90.6 (s).

General procedure for the synthesis of ribonucleoside 3',5'-cyclic phosphonates 25, 26 and 28 via intermediates 22-24. A solution of phosphorylating agent **6** or **7** (0.20 M, 5.5 mL, 1.1 mmol) in dioxane was added to ribonucleoside **8** or **10** (1.0 mmol) which had been dried by repeated coevaporation with anhydrous pyridine (3 x 20 mL). The reaction mixture was stirred for 5 min (in case of **6**) and 15 min (in case of **7**) at 20°C. *N*-Methylimidazole (0.40 mL, 5.0 mmol) was added and the reaction mixture was stirred for 1 h (in case of **6**) and 3 h (in case of **7**) at 20°C. The reaction mixture was diluted with CH_2Cl_2 (50 mL) containing a few drops of TEAB buffer (1 M) and washed twice with TEAB buffer (1 M, 50 mL; 0.1 M, 50 mL). The organic layer was dried ($MgSO_4$), concentrated to a small volume, coevaporated with toluene (2 x 50 mL) and finally evaporated with CH_2Cl_2 (1 x 50 mL) to a colourless foam. Crude product, thus obtained, was purified by short column chromatography using a gradient of CH_3OH in CH_2Cl_2.

2'-*O*-Tetrahydropyranyl-uridine 3',5'-cyclic methylphosphonate (25). 3',5'-Cyclic phosphonate **25** was prepared by phosphonylation of **8** (high running diastereoisomer, 328 mg, 1.0 mmol) with **6** (0.2 M, 5.5 mL, 1.1 mmol) as described above. Crude **25** was purified by short column chromatography applying a 0 to 7% gradient of CH_3OH. Yield: 151 mg (0.39 mmol, 39%); R_f 0.34 ;^1H NMR ($CDCl_3$): δ 1.74 (d, J_{HP} = 17.9 Hz, 3H, P-CH_3), 1.79-2.01 (m, 6H, THP), 3.67-4.86 (m, 5.05 (bs, 1H, THP), 5.73 (d, $J_{5,6}$ = 8.1 Hz, H-5), 5.80 (s, 1H, H-1'), 7.51 (d, $J_{5,6}$ = 8.1 Hz, H-6); ^{13}C NMR (CH_2Cl_2): δ 10.9 (d, J_{CP} = 144 Hz, P-CH_3), 18.5 (s, THP), 30.0 (s, THP), 31.8 (s, THP), 61.9 (s, THP), 68.2 (d, J_{CP} = 8.8 Hz, C-5'), 70.2 (d, J_{CP} = 7.3 Hz, C-3'), 71.3 (d, J_{CP} = 4.4 Hz, C-2'), 73.8 (d, J_{CP} = 4.4 Hz, C-4'), 91.4 (s, C-1'), 96.4 (s, THP), 100.2 (s, C-5), 140.9 (s, C-6), 150.1 (s, C-2), 163.5 (s, C-4); ^{31}P NMR (CH_2Cl_2): δ 34.2 (s).

2'-*O*-Tetrahydropyranyl-*N*-6-levulinoyl-adenosine 3',5'-cyclic methylphosphonate (26). 3',5'-Cyclic phosphonate **26** was prepared by phosphonylation of **10** (high running diastereoisomer, 337 mg, 0.75 mmol) with **6** (0.2 M, 3.75 mL, 0.83 mmol) as described above. Crude **26** was purified by short column chromatography applying a 0 to 5% gradient of CH_3OH. Yield: 176 mg (0.35 mmol, 46%); R_f 0.54; ^1H NMR ($CDCl_3$): δ 1.59-1.92 (m, 6H, THP), 1.82 (d, J_{HP} = 18.1 Hz, 3H, P-CH_3), 2.25 (s, 3H, CH_3 [lev]), 2.96 (t, J = 6.7 Hz, 2H, C$\underline{H}_2$C(=O)CH_3 [lev]), 3.25 (t, J = 6.7 Hz, 2H, C$\underline{H}_2$C(=O)NH [lev]), 3.45 (m, 1H, THP), 3.78 (m, 1H, THP), 4.31-4.92 (m, 5H, H-2', H-4', H-5', H-5'' and THP), 5.41 (m, 1H, H-3'), 6.28 (s, 1H, H-1'), 8.55 (s, 1H, H-2), 8.75 (s, 1H, H-8); ^{13}C NMR ($CDCl_3$): δ 10.9 (d, J_{CP} = 143.5 Hz, P-CH_3), 18.4 (s, THP), 29.8 (s, CH_3 [lev]), 31.7 (s, $\underline{C}H_2$-C(=O)-CH_3 [lev]), 37.5 (s, $\underline{C}H_2$-C(=O)NH [lev]), 61.6 (s, THP), 68.1 (d, J_{CP} = 4.4 Hz, C-5'), 71.7 (d, J_{CP} = 4.4 Hz, C-3'), 73.4 (d, J_{CP} = 5.9 Hz, C-2'), 74.5 (d, J_{CP} = 5.9 Hz, C-4'), 89.6 (s, C-1'), 96.2 (s, THP), 121.9 (s, C-5), 141.2 (s, C-8), 149.4 (s, C-6), 150.4 (s, C-4), 152.4 (s, C-2), 172.4 (s, NH-C=O [lev]), 207.3 (s, CH_3-$\underline{C}$=O [lev]); ^{31}P NMR (CH_2Cl_2): δ 32.7 (s).

2'-*O*-Tetrahydropyranyl-adenosine 3',5'-cyclic methylphosphonate (27). Compound **27** (127 mg, 0.25 mmol) was treated and worked up as described above for **18**. Crude **27** was purified by short column chromatography, applying a 0 to 7% gradient of CH_3OH. Yield: 90 mg (0.22 mmol, 89%); R_f 0.37; ^1H NMR ($CDCl_3$): δ 1.46-1.91 (m, 6H, THP), 1.74 (d, J_{HP} = 18.2 Hz, 3H, P-CH_3), 3.51 (m, 1H, THP), 3.91 (m, 1H, THP), 4.31-4.74 (m, 3H, H-4', H-5' and H-5''), 4.86 (d, $J_{2',3'}$ = 5.7 Hz, 1H, H-2'), 5.11-5.30 (m, 2H, H-3' and THP), 6.09 (s, 1H, H-1'), 6.11 (s, 2H, NH_2), 7.88 (s, 1H, H-2), 8.33 (s, 1H, H-8); ^{13}C NMR ($CDCl_3$): δ 11.2 (d, J_{CP} = 145.0 Hz, P-CH_3), 18.4 (s, THP), 25.0 (s, THP), 30.0 (s, THP), 61.6 (s, THP), 68.1 (d, J_{CP} = 7.3 Hz, C-5'), 71.6 (d, J_{CP} = 4.4 Hz, C-2'), 73.6 (d, J_{CP} = 5.9 Hz, C-4'), 74.5 (d, J_{CP} = 7.3 Hz, C-3'), 89.9 (s, C-1'), 96.2 (s, THP), 119.5 (s, C-5), 138.0 (s, C-8), 148.8 (s, C-4), 153.2 (s, C-2), 155.7 (s, C-6); ^{31}P NMR (CH_2Cl_2): δ 32.3 (s).

2'-*O*-Tetrahydropyranyl-uridine 3',5'-cyclic phenylphosphonate (28). 3',5'-Cyclic phosphonate **28** was prepared by phosphonylation of **8** (high running diastereoisomer, 328 mg, 1.0 mmol) with **7** (0.2 M, 5.5 mL, 1.1 mmol) as described above. Crude **28** was purified by short column chromatography applying a 0 to 5% gradient of CH_3OH. Yield: 320 mg (0.71 mmol, 71%); R_f 0.47; ^1H NMR ($CDCl_3$): δ 1.32-1.84 (m, 6H, THP), 3.40 (m, 1H, THP), 3.78 (m, 1H, THP), 4.49-5.10 (m, 5H, H-2', H-3', H-4', H-5' and H-5''), 5.22 (m, 1H, THP), 5.74 (d, $J_{5,6}$ = 8.2 Hz, 1H, H-5), 5.97 (s, 1H, H-1'), 7.43-8.01 (m, 6H, H-6 and arom. H's [phenyl]); ^{13}C NMR ($CDCl_3$): δ 17.8 (s, THP), 24.8 (s, THP), 29.7 (s, THP), 60.5 (s, THP), 68.3 (d, J_{CP} = 8.8 Hz, C-5'), 71.6 (d, J_{CP} < 1 Hz, C-2'), 73.4 (d, J_{CP} < 1 Hz, C-4'), 74.7 (d, J_{CP} = 5.9 Hz, C-3'), 91.2 (s, C-1'), 95.4 (s, THP), 102.0 (s, C-5), 126.8, 128.0, 128.4, 131.9, 132.1, 133.4 (C arom. [phenyl]), 139.6 (s, C-6), 149.8 (s, C-2), 163.5 (s, C-4); ^{31}P NMR (CH_2Cl_2): δ 19.3 (s).

General procedure for the removal of the 2'-O-tetrahydropyranyl group from nucleoside 3',5'-cyclic methylphosphon(othio)ates and the corresponding phenylphosphon(othio)ates. 2'-O-Tetrahydropyranyl-ribonucleoside 3',5'-cyclic methylphosphon(othio)ate or phenylphosphon(othio)ate (0.5 mmol) was dissolved in 25 mL acetic acid/water [8/2, v/v for methylphosphon(othio)ates and 9/1, v/v for phenylphosphon(othio)ates]. The pH of the solution was brought to 2.0 for the methylderivatives and to 1.0 for the phenylderivatives with acetic acid and the resulting solution was stirred for 36 h at 20 °C. The reaction mixture was neutralized with aqueous ammonia, evaporated under reduced pressure, coevaporated with dioxane (3 x 25 mL) and finally

evaporated with CH_3OH. Crude unprotected nucleoside 3',5'-cyclic methylphosphon(othio)ates or phenylphosphon(othio)ates were purified by column chromatography applying a gradient of CH_3OH in CH_2Cl_2. The fractions containing pure 3',5'-cyclic product were collected and the solvent removed.

Uridine 3',5'-cyclic methylphosphonothioate (4).[9] Compound **15** (150 mg, 0.37 mmol) was treated with acetic acid/water at pH 2.0 and worked up as described above. Crude **4** was taken up in CH_2Cl_2/CH_3OH (5 mL, 2:1, v/v) and applied to a column of Sephadex LH-20 suspended and eluted in the same solvent mixture. The fractions containing pure **4** were collected and evaporated to afford a colourless powder. Yield: 99 mg (0.31 mmol, 83%); R_f 0.35; ^1H NMR (D_2O): δ 1.87 (d, J_{HP} = 15.8 Hz, 3H, P-CH_3), 4.26-4.89 (m, 5H, H-2', H-3', H-4', H-5' and H-5''), 5.75 (s, 1H, H-1'), 5.84 (d, J = 8.0 Hz, 1H, H-5), 7.74 (d, J = 8.0 Hz, 1H, H-6); ^{13}C NMR (D_2O): δ 21.4 (d, J_{CP} = 109.9 Hz, P-CH_3), 68.0 (d, J_{CP} = 7.3 Hz, C-5'), 72.7 (d, J_{CP} = 4.4 Hz, C-2'), 73.0 (d, J_{CP} = 7.3 Hz, C-3'), 75.7 (d, J_{CP} = 4.4 Hz, C-4'), 96.2 (s, C-1'), 103.1 (s, C-5), 143.3 (s, C-6), 151.6 (s, C-2), 165.9 (s, C-4); ^{31}P NMR (CH_2Cl_2/CH_3OH): δ 103.1 (s).

Adenosine 3',5'-cyclic methylphosphonothioate (20). Compound **18** (0.19 g, 0.45 mmol) was treated with acetic acid/water at pH 2.0 and worked up as described above. Crude **20** was purified by short column chromatography applying a 0 to 8% gradient of CH_3OH. The fractions containing pure **20** were collected and evaporated to afford a colourless powder. Yield: 121 mg (0.35 mmol, 78%); R_f 0.16; ^1H NMR ($CDCl_3/CD_3OD$): δ 1.84 (d, J_{HP} = 15.8 Hz, 3H, P-CH_3), 4.08-4.24 (m, 2H, H-5' and H-5''), 4.36-4.52 (m, 2H, H-2' and H-4'), 5.04 (m, 1H, H-3'), 5.77 (s, 1H, H-1'), 7.82 (s, 1H, H-2), 8.04 (s, 1H, H-8); ^{13}C NMR ($CDCl_3/CD_3OD$): δ 21.1 (d, J_{CP} = 109.9 Hz, P-CH_3), 67.5 (d, J_{CP} = 7.4 Hz, C-5'), 72.1 (d, J_{CP} = 4.4 Hz, C-2'), 72.8 (d, J_{CP} = 7.6 Hz, C-3'), 74.7 (d, J_{CP} = 4.4 Hz, C-4'), 92.3 (s, C-1'), 118.7 (s, C-5), 137.4 (s, C-8), 149.2 (s, C-4), 153.4 (s, C-2), 156.2 (s, C-6); ^{31}P NMR (CH_2Cl_2/CH_3OH): δ 103.4 (s).

Uridine 3',5'-cyclic phenylphosphonothioate (21). Compound **19** (233 mg, 0.5 mmol) was treated with acetic acid/water at pH 1.0. After stirring for 36 h at 20°C, crystalline **21** was isolated by filtration. Yield: 139 mg (0.36 mmol, 73%); R_f 0.41; ^1H NMR ($(CD_3)_2SO/CD_3OD$): δ 4.26-5.11 (m, 5H, H-2', H-3', H-4', H-5' and H-5''), 5.70 (s, 1H, H-1'), 5.72 (d, $J_{5,6}$ = 7.4 Hz, 1H, H-5), 7.55-8.11 (m, 6H, H-6 and arom. H's [phenyl]); ^{13}C NMR ($(CD_3)_2SO/CD_3OD$): δ 69.1 (d, J_{CP} = 7.3 Hz, C-5'), 72.7 (d, J_{CP} = 5.9 Hz, C-2'), 72.9 (d, J_{CP} = 7.3 Hz, C-3'), 76.7 (d, J_{CP} = 4.4 Hz, C-4'), 96.5 (s, C-1'), 103.2 (s, C-5), 129.6, 129.9, 132.9, 133.2 (C-arom. [phenyl]), 143.8 (s, C-6), 151.6 (s, C-2), 165.6 (s, C-4); ^{31}P NMR (CH_3OH): δ 90.6 (s).

Uridine 3',5'-cyclic methylphosphonate (3). Compound **25** (194 mg, 0.5 mmol) was treated with acetic acid/water at pH 2.0 and worked up as described above. Crude **3** was taken up in CH_2Cl_2/CH_3OH (5 mL, 2:1, v/v) and applied to a column of Sephadex LH-20 suspended and eluted in the same solvent mixture. The fractions containing pure **3** were collected and evaporated to afford a colourless powder. Yield: 125 mg (0.41 mmol, 82%); R_f 0.18; ^1H NMR (D_2O): δ 1.87 (d, J = 18.3 Hz, 3H, P-CH_3), 4.34-4.80 (m, 5H, H-2', H-3', H-4', H-5' and H-5''), 5.82 (s, 1H, H-1'), 5.92 (d, J = 8.1 Hz, 1H, H-5), 7.76 (d, J = 8.1 Hz, 1H, H-6); ^{13}C NMR (D_2O): δ 11.0 (d, J_{CP} = 142 Hz, P-CH_3), 69.7 (d, J_{CP} = 8.8 Hz, C-5'), 71.5 (d, J_{CP} = 5.9 Hz, C-4'), 72.1 (d, J_{CP} = 7.3 Hz, C-3'), 75.5 (d, J_{CP} = 5.9 Hz, C-2'), 95.4 (s, C-1'), 103.0 (s, C-5), 143.5 (s, C-6), 152.5 (s, C-2), 167.8 (s, C-4); ^{31}P NMR (CH_2Cl_2/CH_3OH): δ 37.5 (s).

Adenosine 3',5'-cyclic methylphosphonate (29). Compound **27** (90 mg, 0.22 mmol) was treated with acetic acid/water at pH 2.0 and worked up as described above. Crude **29** was purified by column chromatography applying a 0 to 8% gradient of CH_3OH. The fractions containing pure **27** were collected and evaporated to afford a colourless powder. Yield: 55.2 mg (0.17 mmol, 77%); R_f 0.14; ^1H NMR ($CDCl_3/CD_3OD$): δ 1.75 (d, J_{HP} = 18.3 Hz, 3H, P-CH_3), 4.30-4.85 (m, 4H, H-2', H-4', H-5' and H-5''), 5.32 (m, 1H, H-3'), 6.06 (s, 1H, H-1'), 8.17 (s, 1H, H-2), 8.25 (s, 1H, H-8); ^{13}C NMR ($CDCl_3/CD_3OD$): δ 9.9 (d, J_{CP} = 145.0 Hz, P-CH_3), 68.2 (d, J_{CP} = 7.3 Hz, C-5'), 70.6 (d, J_{CP} = 4.4 Hz, C-2'), 71.6 (d, J_{CP} = 7.3 Hz, C-3'), 74.7 (d, J_{CP} = 5.9 Hz, C-4'), 91.6 (s, C-1'), 100.2 (s, C-5), 139.3 (s, C-8), 148.4 (s, C-4), 152.4 (s, C-2), 155.3 (s, C-6); ^{31}P NMR (CH_2Cl_2/CH_3OH): δ 35.2 (s).

Uridine 3',5'-cyclic phenylphosphonate (30). Compound **28** (225 mg, 0.5 mmol) was treated with acetic acid/water at pH 1.0 and worked up as described above. Crude **30** was purified by column chromatography applying a 0 to 7% gradient of CH_3OH. The fractions containing pure **30** were collected and evaporated to afford a colourless powder. Yield: 126 mg (0.34 mmol, 69%); R_f 0.30; ^1H NMR ($(CD_3)_2SO/CD_3OD$): δ 4.23-4.75 (m, 6H, H-2', H-3', H-4', H-5' and H-5''), 5.65 (d, J = 7.4 Hz, 1H, H-5), 5.67 (s, 1H, H-1'), 7.49-7.85 (m, 6H, H-6 and arom. H's [phenyl]); ^{13}C NMR ($(CD_3)_2SO/CD_3OD$): δ 69.8 (d, J_{CP} = 8.8 Hz, C-5'), 71.2 (d, J_{CP} = 4.4 Hz, C-2'), 71.7 (d, J_{CP} = 7.3 Hz, C-3'), 76.1 (d, J_{CP} = 4.4 Hz, C-4'), 95.2 (s, C-1'), 102.9 (s, C-5), 124.4, 129.6, 129.8, 132.6, 132.8, 134.7 (C arom. [phenyl]), 143.1 (s, C-6), 150.8 (s, C-2), 164.5 (s, C-4); ^{31}P NMR (CH_2Cl_2/CH_3OH): δ 20.7 (s).

Adenosine 3'-methylmethoxyphosphonothioate (33) and adenosine 5'-methylmethoxyphosphonothioate (34). Compound **16** (0.47 g, 0.88 mmol) was dissolved in NH_3/CH_3OH (50 mL, half-saturated at 0°C) and the resulting solution was kept in a carefully sealed flask at 20°C. TLC analysis and ^{31}P NMR spectroscopy after 6 h revealed conversion of **16** into two new products (δp 99.4 and 97.6 ppm) with identical R_f-value (0.44). The reaction mixture was evaporated to dryness and the residue was purified by short column chromatography applying a 0 to 4% gradient of CH_3OH. The unseparable products **31** and **32** were isolated in a total yield of 90%. Compounds **31** and **32** were dissolved in acetic acid/water (4/1, v/v, 25 mL) and the resulting solution was stirred for 24 h at 20°C. The reaction mixture was evaporated under reduced pressure, coevaporated with dioxane (3 x 50 mL) and finally evaporated with CH_3OH (2 x 50 mL). Crude **33** and **34** were purified by short column chromatography applying a 0 to 7% gradient of CH_3OH and subsequently characterized by NMR spectroscopy. Yield **33** (based on **16**): 0.12 g (0.32 mmol, 40%); R_f 0.37; ^1H NMR ($CDCl_3/CD_3OD$): δ 1.83 (d, J_{HP} = 15.4 Hz, 3H, P-CH_3), 3.71 (d, J_{HP} = 13.8 Hz, 3H, P-OCH_3), 4.23-4.48 (m, 4H, H-3', H-4', H-5' and H-5''), 4.55 (dd, $J_{1',2'}$ = 3.8 Hz, $J_{2',3'}$ = 5.1 Hz, 1H, H-2'), 6.03 (d, $J_{1',2'}$ = 3.8 Hz, 1H, H-1'), 8.19 (s, 1H, H-2), 8.25 (s, 1H, H-8); ^{13}C NMR ($CDCl_3/CD_3OD$): δ 21.0 (d, J_{CP} = 110 Hz, P-CH_3), 63.8 (d, J_{CP} = 5.7 Hz, P-OCH_3), 66.6 (s, C-5'), 72.1 (d, J_{CP} = 4.4 Hz, C-2'), 73.5 (d, J_{CP} = 7.6 Hz, C-3'), 74.8 (d, J_{CP} = 4.4 Hz, C-4'), 92.7 (s, C-1'), 118.6 (s, C-5), 139.1 (s, C-8), 147.2 (s, C-4), 152.8 (s, C-2), 155.0 (s, C-6); ^{31}P NMR (CH_2Cl_2/CH_3OH): δ 99.6 (s). Yield **34** (based on **16**): 0.10 g (0.27 mmol, 34%); R_f 0.41; ^1H NMR ($CDCl_3/CD_3OD$): δ 1.93 (d, J_{HP} = 15.6 Hz, 3H, P-CH_3), 3.77 (d, J_{HP} = 13.7 Hz, 3H, P-OCH_3), 3.91 (t, $J_{4',5'}$ = $J_{5',5''}$ = 12.4 Hz, 1H, H-5'), 3.92 (t, $J_{4',5''}$ = $J_{5',5''}$ = 12.4 Hz, 1H, H-5''), 4.41 (m, 1H, H-4'), 5.03 (dd, $J_{1',2'}$ = 7.9 Hz, $J_{2',3'}$ = 5.1 Hz, 1H, H-2'), 5.25 (dd, $J_{2',3'}$ = 5.1 Hz, $J_{3',4'}$ = 12.9 Hz, 1H, H-3'), 5.90 (d, $J_{1',2'}$ = 7.9 Hz, 1H, H-1'), 8.01 (s, 1H, H-2), 8.17 (s, 1H, H-8); ^{13}C NMR ($CDCl_3/CD_3OD$): δ 21.0 (d, J_{CP} = 111 Hz, P-CH_3), 64.1 (d, J_{CP} = 5.6 Hz, P-OCH_3), 67.5 (d, J_{CP} = 7.2 Hz, C-5'), 71.8 (s, C-2'), 72.7 (s, C-3'), 74.9 (d, J_{CP} = 4.5 Hz, C-4'), 92.0 (s, C-1'), 119.3 (s, C-5), 138.0 (s, C-8), 148.0 (s, C-4), 152.8 (s, C-2), 155.9 (s, C-6); ^{31}P NMR (CH_2Cl_2/CH_3OH): δ 98.6 (s).

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