Synthesis and Enzymatic Deprotection of Biodegradably Protected Dinucleoside-2′,5′-monophosphates: 3-(Acetyloxy)-2,2-bis(ethoxycarbonyl)propyl Phosphoesters of 3′-*O*-(Acyloxymethyl)adenylyl-2′,5′-adenosines

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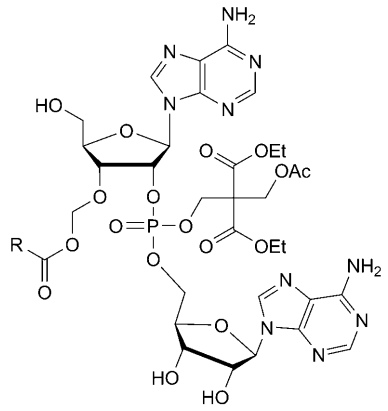
As a first step towards a viable prodrug strategy for short oligoribonucleotides, such as 2–5A and its congeners, adenylyl-2′,5′-adenosines bearing a 3-(acetyloxy)-2,2-bis(ethoxycarbonyl)propyl group at the phosphate moiety, and an (acetyloxy)methyl- or a (pivaloyloxy)methyl-protected 3′-OH group of the 2′-linked nucleoside have been prepared. The enzyme-triggered removal of these protecting groups by hog liver carboxyesterase at pH 7.5 and 37° has been studied. The (acetyloxy)methyl group turned out to be too labile for the 3′-*O*-protection, being removed faster than the phosphate-protecting group, which results in 2′,5′- to 3′,5′-isomerization of the internucleosidic phosphoester linkage. In addition, the starting material was unexpectedly converted to the 5′-*O*-acetylated derivative. (Pivaloyloxy)methyl group appears more appropriate for the purpose. The fully deprotected 2′,5′-ApA was accumulated as a main product, although, even in this case, the isomerization of the starting material takes place.

Introduction. – The 5′-triphosphates of 2′,5′-oligoadenylates, collectively known as 2–5A, are activators of an intracellular endoribonuclease RNase L, which cleaves both messenger and ribosomal RNA, resulting in apoptosis [1][2]. The formation of 2–5A is triggered by interferon in response to various stimuli, including viral infection. Interferon induces 2–5A synthetase that, upon activation by double-stranded RNA, converts ATP into 2′,5-linked oligoribonucleotides, and 2–5A phosphodiesterase then produces 2–5A from longer oligomers [3]. Accordingly, activation of RNase L by synthetic 2–5A might offer a way to combat against viral diseases. In addition, 2–5A activation has been reported to result in apoptosis in metastatic prostate cell lines [4].

The major limitations of 2–5A for therapeutic applications include short biological half-life and ionic structure that leads to insufficient internalization to cytoplasm [3]. Numerous structurally modified analogues of 2–5A have been prepared to increase the stability in serum and cytoplasm [5–12], but no prodrug strategy for the enhancement of cellular uptake has been introduced. In principle, masking of the negative charges of the sugar-phosphate backbone with biodegradable lipophilic protecting groups should improve the internalization, but the presence of the 3′-OH groups complicates the situation.

The attack of the neighboring OH group on the protected phosphodiester linkage is extremely fast, resulting in both 2′ → 3′ phosphate migration and cleavage of the P-O5′ bond [13]. Accordingly, the 3′-OH functions must also be protected, and these

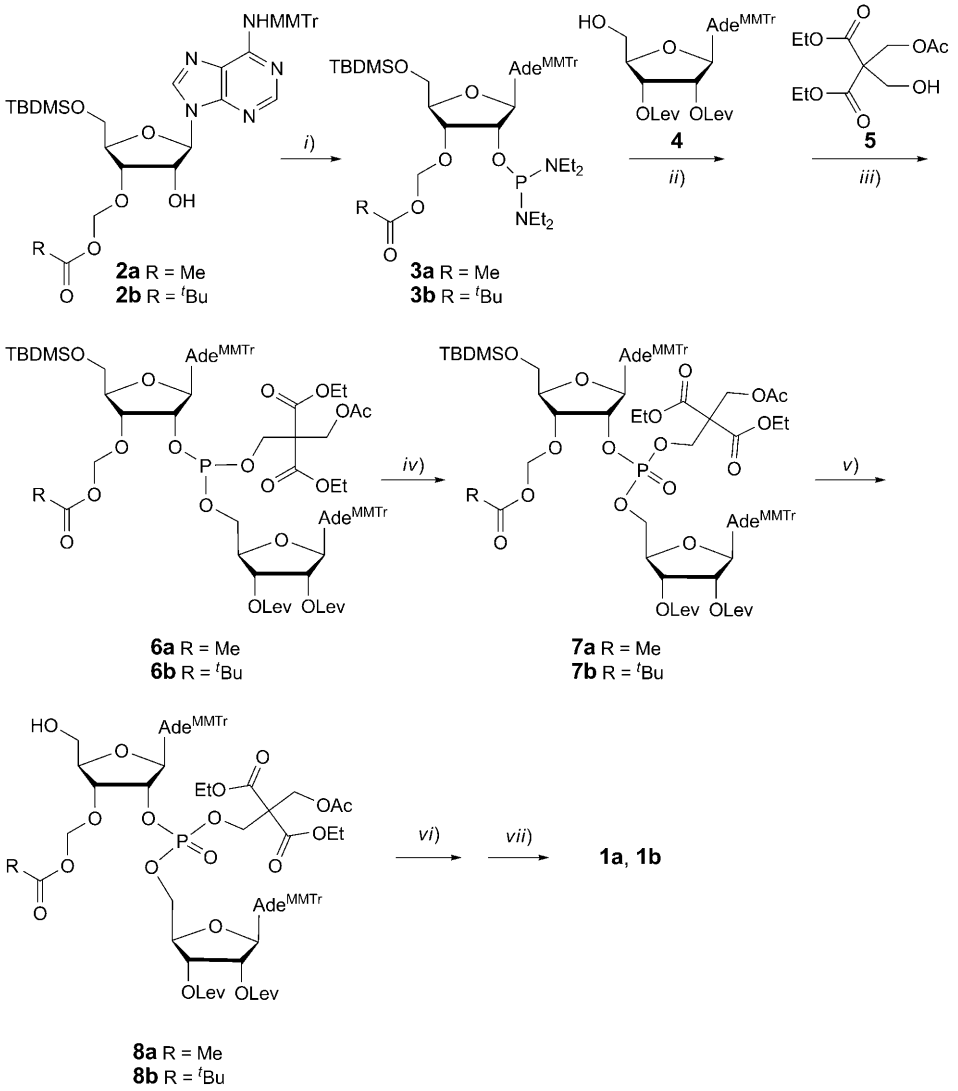
protecting groups should be removed more slowly than the protecting groups of the phosphodiester linkages. As a first step towards a viable prodrug strategy for 2–5A, we now report on our studies on a dimeric model compound, *viz.* adenylyl-2′,5′-adenosine, which has both the phosphodiester linkage and the 3′-OH group of the 2′-linked nucleoside protected with an esterase-labile protecting group. For the phosphate protection, 3-(acetyloxy)-2,2-bis(ethoxycarbonyl)propyl group [14] has been used. This group undergoes esterase-catalyzed deacetylation, and the remnants of the group are removed by *retro*-aldol condensation and concomitant phosphate elimination. Two different esterase-labile groups, *viz.* a (pivaloyloxy)methyl and an (acetyloxy)methyl ($AcOCH_2$) group, have been used for the protection of the neighboring OH function to find out whether a successful deprotection strategy could be based on one of these groups. As mentioned above, the phosphate-protecting group should be removed before exposure of the 3′-OH group, and the enzymatic removal of the (pivaloyloxy)-methyl group expectedly is slower than the removal of the $AcOCH_2$ group. For this purpose, protected dinucleoside-2′,5′-monophosphates **1a** and **1b** have been prepared, and their deprotection by hog liver carboxyesterase (HLE) has been studied.



1a R = Me
1b R = ^{t}Bu

Results and Discussion. – *Syntheses.* 3-(Acetyloxy)-2,2-bis(ethoxycarbonyl)propyl protected 3′-*O*-(acyloxymethyl)adenylyl-2′,5′-adenosines, **1a** and **1b**, were assembled from the appropriately protected nucleosides and 2-(acetyloxymethyl)-2-(hydroxymethyl)malonate as outlined in *Scheme 1*. Accordingly, the 2′-OH function of 3′-*O*-(acetyloxymethyl)-N^6-(4-methoxytrityl)adenosine (**2a**) or its 3′-*O*-(pivaloyloxymethyl) counterpart (**2b**) was first phosphitylated with bis(diethylamino)phosphorochloridite ($(Et_2N)_2PCl$) in the presence of Et_3N, and the remaining Et_2N ligands were then sequentially replaced with 2′,3′-di-*O*-levulinoyl-N^6-(4-methoxytrityl)adenosine (**4**) and diethyl 2-(acetyloxymethyl)-2-(hydroxymethyl)malonate (**5**) using 1*H*-tetrazole as an activator. The resulting phosphite triesters **6a** and **6b** were oxidized to phosphate esters **7a** and **7b**, respectively, with I_2 in aqueous THF containing 2,6-lutidine. The course of the phosphitylation, coupling, and oxidation steps described above were monitored by ^{31}P-NMR spectroscopy. The formation of *N*,*N*,*N*′,*N*′-tetraethylphosphorodiamidites, **3a**

Scheme 1



i) $(Et_2N)_2PCl$, Et_3N, CH_2Cl_2. *ii*) 1*H*-Tetrazole (TetH), MeCN. *iii*) TetH, MeCN. *iv*) I_2, THF, H_2O, 2,6-lutidine. *v*) Bu_4NF, AcOH, THF. *vi*) NH_2NH_3OAc, MeOH, CH_2Cl_2 for **8a**; NH_2NH_2, AcOH, pyridine for **8b**. *vii*) 80% AcOH.

and **3b**, was accompanied by appearance of a ^{31}P-NMR resonance at 137.6 and 137.2 ppm, respectively. The phosphite triester **6a** resonated at 140.7 and 140.5 ppm ((R_P)- and (S_P)-diastereoisomers, resp.) and **6b** at 140.6 and 140.5 ppm, and the phosphate triesters, **7a** and **7b**, at −2.4 and −2.5, and −2.4 and −2.6 ppm, respectively. The silyl group of the fully protected dimer **7b** was removed with Bu_4NF

in the presence of AcOH to yield **8b**. Compound **7a** was desilylated to **8a** with $Et_3N \cdot 3$ HF, to keep the $AcOCH_2$ group intact [15]. The levulinoyl groups were finally removed with NH_2NH_3OAc and the 4-methoxytrityl groups with aqueous AcOH, to give **1a** and **1b** as a mixture of (R_P)- and (S_P)-diastereoisomers. *Fig. 1* shows the RP-HPLC traces of the purified products **1a** and **1b**.

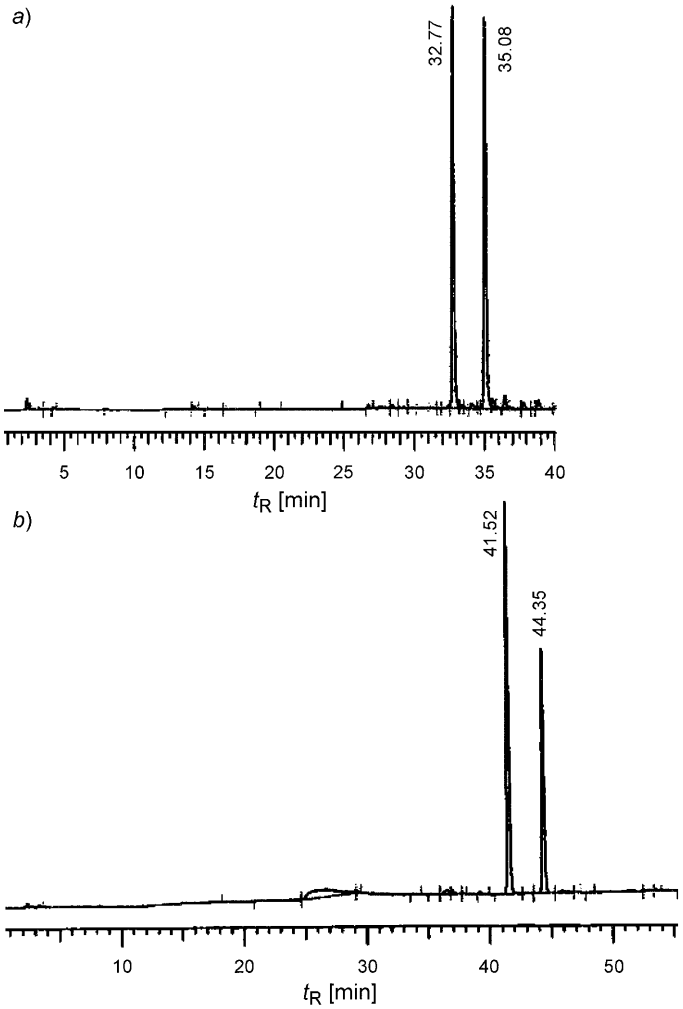
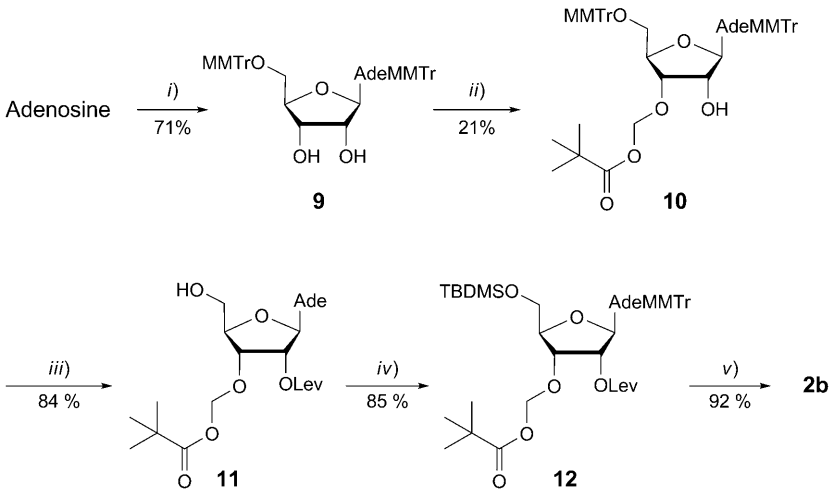


Fig. 1. *Analytical RP-HPLC traces of* a) *the two diastereoisomers of 3-(acetyloxy)-2,2-bis(ethoxycarbonyl)propyl-protected 3′-*O*-(acyloxymethyl)adenylyl-2′,5′-adenosines* **1a** (t_R 32.77 and 35.08 min) *and* b) **1b** (t_R 41.52 and 44.35 min) *at 260 nm. Thermo ODS Hypersil C18 column* (4 × 250 mm, 5 μm; flow rate 0.95 ml min^{-1}); buffer: AcOH/AcONa 0.045 : 0.015 mol l^{-1}), containing NH_4Cl (0.1 mol l^{-1}); buffer *A:* buffer in 2% MeCN, buffer *B:* buffer in 60% MeCN. Isocratic elution with *A* for 5 min and gradient elution from 0 to 71% *B* in 45 min.

5′-*O*-[(*tert*-Butyl)(dimethyl)silyl]-N^6-(4-methoxytrityl)-3′-*O*-(pivaloyloxymethyl)-adenosine (**2b**) used for the assembly of the corresponding phosphate-protected dinucleoside monophosphate, **1b**, was obtained as depicted in *Scheme 2*. Adenosine was converted to its 5′-*O*,N^6-bis(4-methoxytrityl) derivative **9** [16] and subjected to alkylation with (pivaloyloxy)methyl chloride to obtain a mixture of 2′-*O*- and 3′-*O*-(pivaloyloxymethyl) derivatives. The latter reaction turned out to be somewhat difficult. The best yield was obtained by deprotonating the OH group with 1 equiv. of NaH in THF and then treating the nucleoside with (pivaloyloxy)methyl chloride in the presence of a catalytic amount of NaI. Even then the 3′-*O*-alkylated product **10** was obtained only in a 21% total yield. The yield of the undesired 2′-*O*-alkylated product was 7%. Unexpectedly, a significant amount of 3′-*O*- and 2′-*O*-pivalates was formed, which explains the low yield of the pivaloyloxymethylated products. The 2′-OH function of **10** was then protected with a levulinoyl group, and the 4-methoxytrityl groups were removed with acid to obtain **11**. The exposed 5′-OH function was protected with a ^{t}BuMe$_2$Si group and the 6-amino group with a 4-methoxytrityl group to give compound **12**. Removal of the levulinoyl group afforded the desired product **2b**.

Scheme 2

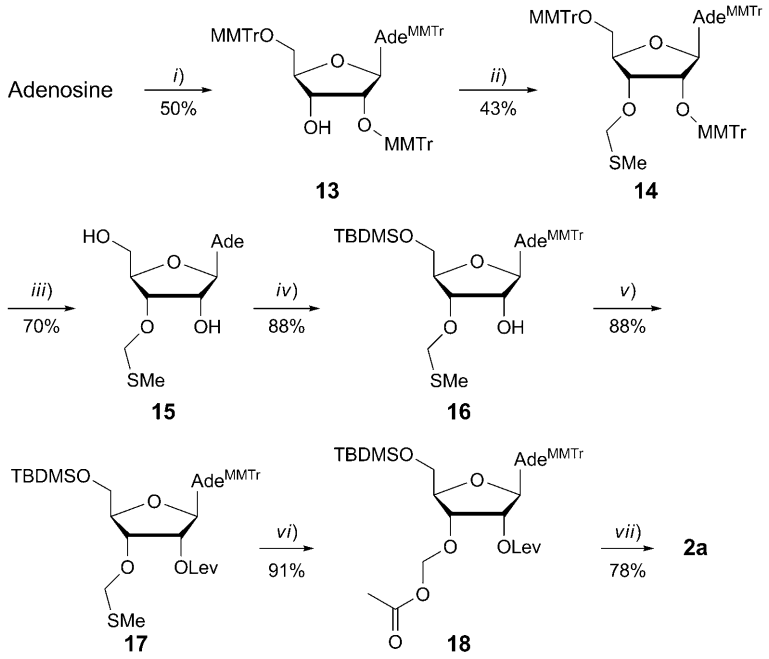


i) Monomethoxytrityl chloride (=(4-methoxyphenyl)(diphenyl)methyl chloride) (MMTrCl), pyridine. *ii*) PivOCH$_2$Cl (=(pivaloyloxy)methyl chloride=(2,2-dimethylpropanoyl)methyl chloride), NaH, NaI, THF. *iii*) 1. Lev$_2$O (Lev=levunoyl=4-oxopentanoyl), dioxane, pyridine; 2. 80% AcOH. *iv*) 1. ^{t}BuMe$_2$SiCl (TBDMSCl), pyridine; 2. MMTrCl, pyridine. *v*) NH_2NH_2, AcOH, pyridine.

Attempts to prepare the corresponding 3′-*O*-(acetyloxymethyl) derivative **2a** in a similar manner failed. This nucleoside was obtained as depicted in *Scheme 3*. Adenosine was converted to 2′-*O*,5′-*O*,N^6-tris(4-methoxytrityl)adenosine (**13**) by treating with 3 equiv. of 4-methoxytrityl chloride in pyridine. Compound **13** was deprotonated with 1 equiv. of NaH and alkylated with MeSCH$_2$Cl to obtain the 3′-*O*-[(methylthio)methyl] derivative **14**. After acid-catalyzed detritylation to **15**, the 5′-*O* was protected with a ^{t}BuMe$_2$Si group, and the N^6-atom with a 4-methoxytrityl group to

yield **16**. 2′-*O* Position was protected with a levulinoyl group to obtain **17**. The $MeSCH_2$ group was transformed to a $ClCH_2$ group with SO_2Cl_2 and then to an $AcOCH_2$ group (→**18**) with AcOK in the presence of dibenzo-18-crown-6. Removal of the levulinoyl group with NH_2NH_3OAc in $MeOH/CH_2Cl_2$ gave **2a**, leaving the $AcOCH_2$ group intact.

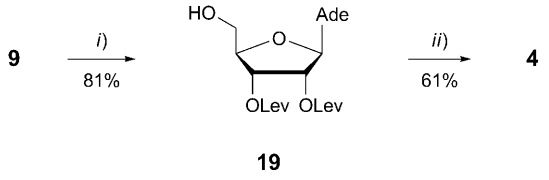
Scheme 3



i) MMTrCl, pyridine. *ii*) 1. NaH, DMF, 2. $MeSCH_2Cl$, DMF. *iii*) 80% AcOH. *iv*) 1. TBDMSCl, pyridine, 2. MMTrCl, pyridine. *v*) Lev_2O, dioxane, pyridine. *vi*) 1. SO_2Cl_2, CH_2Cl_2, 2. AcOK, dibenzo-18-crown-6, CH_2Cl_2. *vii*) H_2NNH_3OAc, CH_2Cl_2, MeOH.

The 5′-linked nucleoside **4** was obtained by protecting the 2′- and 3′-OH groups of 5′-*O*,N^6-bis(4-methoxytrityl)adenosine (**9**) with levulinoyl groups, detritylating the product, and re-introducing the 4-methoxytrityl group at N^6 by using Me_3Si group for a transient protection [17] at 5′-*O* (*Scheme 4*). The preparation of diethyl 2-(acetyloxymethyl)-2-(hydroxymethyl)malonate (**5**) has been described previously [14e].

Scheme 4



i) 1. Lev_2O, dioxane, pyridine, 2. 80% AcOH. *ii*) 1. Me_3SiCl, pyridine, 2. MMTrCl, pyridine, 3. Bu_4NF, AcOH, THF.

Enzymatic Deprotection. The deprotection of protected adenylyl-2′,5′-adenosines **1a** and **1b** was followed in *HEPES* buffer at pH 7.5 and 37° in the presence of hog liver carboxyesterase (HLE; 2.6 units ml^{-1}). The composition of the aliquots withdrawn at appropriate intervals from the mixture was determined by RP-HPLC, and the products were characterized by mass spectrometric analysis (HPLC/ESI-MS) and/or by spiking with authentic samples (in the case of 2′,5′-ApA, 3′,5′-ApA, adenosine, and inosine).

Fig. 2 shows the HPLC traces for the mixture of the enzymatic deprotection of 3′-*O*-(acetyloxymethyl)adenosin-2′-yl adenosin-5′-yl 3-(acetyloxy)-2,2-bis(ethoxycarbonyl) phosphate (**1a**) at various times after the addition of the enzyme. These data show that three initial products are formed in parallel: deacetylated phosphotriester **20a**, phosphate-protected adenylyl-2′,5′-adenosine **21**, and a monoacetylated derivative of **21**, most likely the 5′-*O*-Ac derivative **21-Ac** (*Scheme 5*). Triester **20a** then gives diester **22a** by a HO^- ion-catalyzed loss of HCHO and concomitant elimination of the remnant

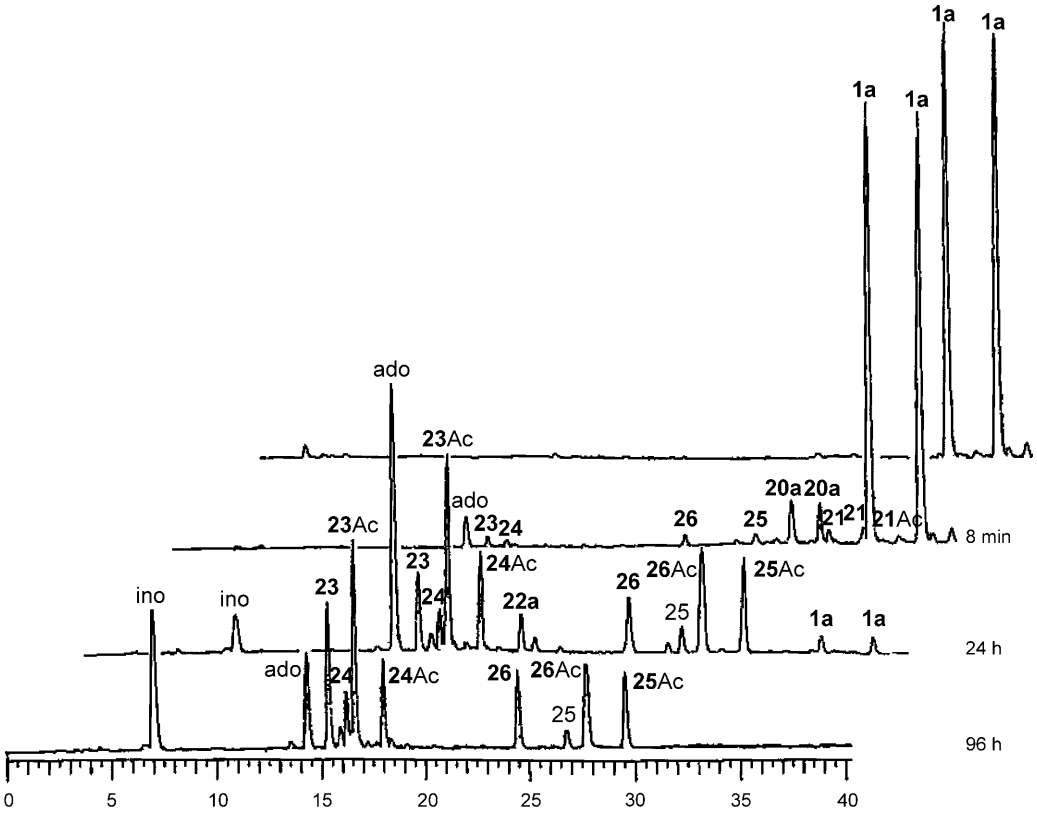
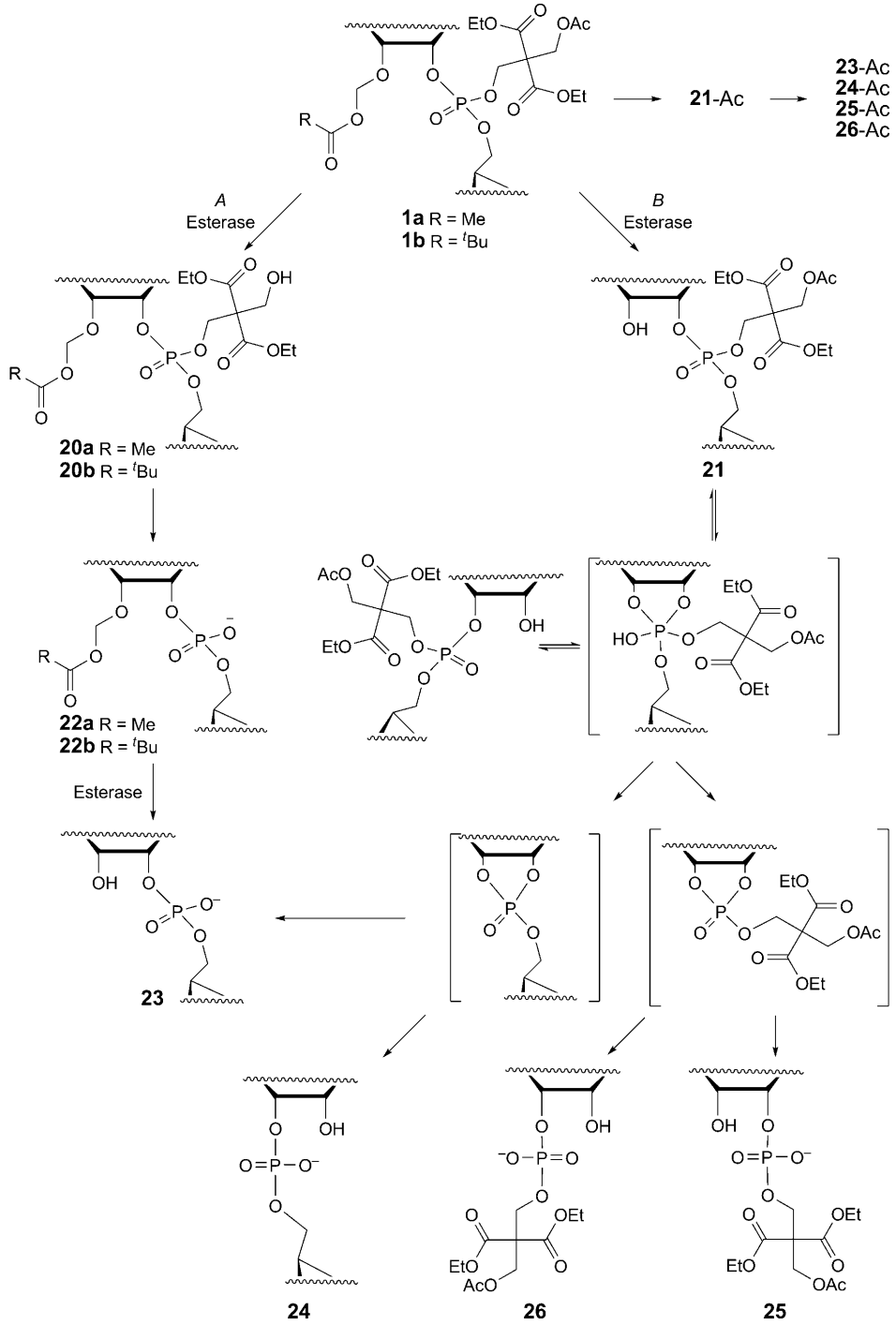


Fig. 2. *RP-HPLC Traces for the HLE-catalyzed hydrolysis of 3′-*O*-(acetyloxymethyl)adenosin-2′-yl adenosin-5′-yl 3-(acetyloxy)-2,2-bis(ethoxycarbonyl)propyl phosphate* (**1a**) *at pH 7.5 and 37.0°* (I = 0.1 mol l^{-1} with NaCl). For the chromatographic conditions, see the *Exper. Part*.

Scheme 5. *HLE-Triggered Deprotection*



of the phosphate-protecting group as diethyl 2-methylidenemalonate [14a]. Finally, enzymatic deacetylation of the 3′-*O*-(acetyloxymethyl) group, followed by rapid hydrolysis of the resulting half-acetal gives the desired product, 2′,5′-ApA (**23**).

The competing route *via* the 3′-*O*-deprotected triester **21** also yields 2′,5′-ApA (**23**), besides several additional products, *viz*. 3′,5′-ApA (**24**), and adenosine 2′-*O*- and 3′-*O*-[3-(acetyloxy)-2,2-bis(ethoxycarbonyl)propyl] phosphates **25** and **26**, respectively. All these compounds are formed *via* a phosphorane intermediate obtained by an attack of the 3′-OH function at the P-atom. This intermediate is decomposed, in addition to acyclic 2′,5′- and 3′,5′-phosphotriesters, to two different 2′,3′-cyclic phosphotriesters obtained by removal of either the phosphate protecting group or the 5′-linked nucleoside. Hydrolysis of these cyclic esters then gives 2′,5′- and 3′,5′-ApA (**23** and **24**, resp.), and diesters **25** and **26**. The mechanisms of the hydrolysis of ribonucleoside 2′- and 3′-phosphotriesters have been discussed previously in more detail [13][18]. Consistent with earlier findings [14e], the enzymatic deacetylation of **25** and **26** is much slower than the deacetylation of triester **1a**, and they are, hence, accumulated as virtually stable products.

In spite of the fact that 2′,5′-ApA (**23**) is formed *via* both of the routes indicated in *Scheme 5*, its proportion in the product mixture remains very low. The main reason is that, in addition to the products mentioned above, monoacetylated derivatives of products **23**–**26** were accumulated in a considerable amount. A likely explanation for this unexpected finding is that the Ac group of the 3′-*O*-(acetyloxymethyl) moiety migrates to the 5′-OH group. Accordingly, parallel to the formation of **22a** and **21**, the starting material is converted to the 5′-*O*-acetylated derivative of **21**, which is then hydrolyzed along the pathway depicted for the breakdown of **21**. As seen from *Fig. 2*, upon 95% disappearance of the diastereoisomeric starting material, the main products were adenosine (25%) and acetylated derivatives of 2′,5′-ApA (8%) and 3′,5′-ApA (15%), and acetylated diesters **25** and **26** (23%). In addition, small amounts of 3′,5′-ApA (**24**; 3%), 2′,5′-ApA (**23**; 6%), diesters **25** and **26** (8%), and intermediate **22a** (3%) were accumulated. Even though this kind of Ac migration is not possible with 2–5A; owing to the absence of free 5′-OH function, the removal of the protecting group at 3′-*O* still appears too facile compared to the phosphate deprotection to afford a viable pro-drug strategy for 2′,5′-ApA (**23**).

To decelerate the removal of the 3′-*O*-protecting group, the $AcOCH_2$ group was replaced with a more bulky (pivaloyloxy)methyl group. *Fig. 3* shows the HPLC traces for the mixture of the enzymatic deprotection of 3′-*O*-(pivaloyloxymethyl)adenosin-2′-yl adenosine-5′-yl 3-(acetyloxy)-2,2-bis(ethoxycarbonyl) phosphate (**1b**). These data reveal that even the 3′-*O*-(pivaloyloxy)methyl group is removed somewhat too fast compared to the removal of the phosphate-protecting group to allow exposure of 2′,5′-ApA (**23**) as the sole product. In fact, deprotection of the 3′-OH function is twice as rapid as the phosphate deprotection, but, since also the former route yields **23** in addition to its 3′,5′-isomer, **24** and diesters **25** and **26**, **23** is the main product of the enzymatic deprotection. As seen from *Fig. 3*, virtually all the starting material, **1b**, had been disappeared in 24 h. At this stage, the product mixture consisted of diesters **22b** (24%), **25** (12%), and **26** (12%) in addition to 2′,5′-ApA (**23**; 20%), 3′,5′-ApA (**24**; 8%), adenosine (16%), and inosine (7%). Owing to slow enzymatic conversion of **22b** to **23**, the proportion of the latter compounds was, however, increased with time, being

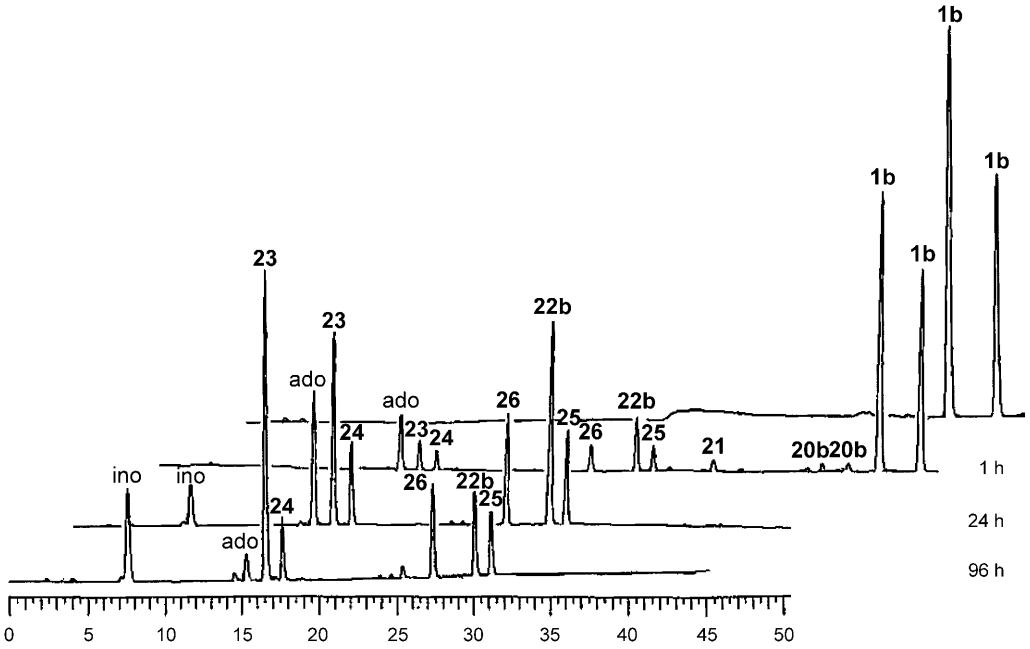


Fig. 3. *RP-HPLC Traces for the PLE-catalyzed hydrolysis of 3′-*O*-(pivaloyloxymethyl)adenosin-2′-yl adenosine-5′-yl 3-(acetyloxy)-2,2-bis(ethoxycarbonyl)propyl phosphate* (**1b**) *at pH 7.5 and 37.0°* (I = 0.1 mol l^{-1} with NaCl). For the chromatographic conditions, see the *Exper. Part*.

finally 37%. Prolonged treatment with HLE also resulted in partial deamination of adenosine to inosine.

Conclusions. – Protected adenylyl-2′,5′-adenosines bearing a 3-(acetyloxy)-2,2-bis(ethoxycarbonyl)propyl group at the phosphate moiety and an (acetyloxy)methyl (*i.e.*, **1a**) or a (pivaloyloxy)methyl group (*i.e.*, **1b**) at the neighboring 3′-*O* have been prepared. During the HLE-catalyzed deprotection of **1a**, the $AcOCH_2$-protected compound was unexpectedly converted to an acetylated one, most likely by migration of the Ac group to 5′-O of the 2′-linked nucleoside and hydrolytic removal of the remaining 3′-*O*-(hydroxymethyl) group. The exposed 3′-OH function readily attacks on the still protected phosphate linkage, resulting in cleavage and isomerization of the 2′,5′-phosphoester linkage to a 3′,5′-linkage. Accordingly, 2′,5′-ApA (**23**) is released only as a minor product. With the 3′-*O*-(pivaloyloxymethyl)-protected compound, **1b**, the desired 2′,5′-ApA (**23**) is the main product, although, even in this case, the departure of the 3′-*O*-(pivaloyloxymethyl) group competes with the deprotection of the phosphate group, leading to partial isomerization and cleavage of the internucleosidic phosphoester linkage.

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Experimental Part

General. Chemicals were purchased from *Sigma–Aldrich*, *Fluka*, and *Merck*. MeCN, CH_2Cl_2, and pyridine were dried over 4-Å molecular sieves. Dioxane was dried over 3-Å molecular sieves. Et_3N was dried by refluxing over CaH_2 and distilled before use. Column chromatography (CC): *Fluka* silica gel *60* (SiO_2; 230–400 mesh). ^{1}H-, ^{13}C-, ^{31}P-, and 2D-NMR spectra: *Bruker Avance 500* NMR spectrometer; the chemical shifts are given in ppm with reference to internal TMS; the coupling constants *J* are given in Hz. LC/ESI-MS: *Perkin-Elmer Sciex-API-365* triple-quadrupole. HPLC: *Merck Hitachi LaChrom D7000* with L-7455 UV-detector and L-7100 pump. HR-ESI-MS: *Bruker Daltonics micrOTOF-Q*.

5′-O,N^6-*Bis[(4-methoxyphenyl)(diphenyl)methyl]adenosine*; **9**). Adenosine (26.2 mmol, 7.00 g) was co-evaporated twice from dry pyridine and dissolved in the same solvent (40 ml). A soln. of 4-methoxytrityl chloride (MMTrCl; 57.7 mmol, 17.8 g) in pyridine (60 ml) was added, and the mixture was stirred overnight at 35°. MeOH (25 ml) was added, and the stirring was continued for 0.5 h. The solvent was removed by evaporation under reduced pressure, and the residual oil was partitioned between H_2O and $CHCl_3$. The org. layer was washed with sat. aq. $NaHCO_3$ and sat. aq. NaCl, dried (Na_2SO_4) and evaporated to dryness. The residue was co-evaporated with toluene and purified by SiO_2 chromatography using CH_2Cl_2 containing 1–2% MeOH as eluent to afford **9** (15.2 g, 71%). Yellowish foam. ^{1}H-NMR (500 MHz, $CDCl_3$): 8.07 (*s*, H–C(8)); 8.02 (*s*, H–C(2)); 7.17–7.39 (*m*, 24 H of MMTr); 7.09 (br. *s*, HN–C(6)); 6.79–6.84 (*m*, 4 H of MMTr); 6.64 (br. *s*, HO–C(2′)); 5.95 (*d*, *J* = 6.0, H–C(1′)); 4.79 (*m*, H–C(2′)); 4.42–4.44 (*m*, H–C(4′)); 4.37 (*dd*, *J* = 5.5, 2.0, H–C(3′)); 3.80 (*s*, 2 MeO of MMTr); 3.70 (br. *s*, HO–C(3′)); 3.49 (*dd*, *J* = 10.5, 3.5, 1 H of CH_2(5′)); 3.25 (*dd*, *J* = 10.5, 3.5, 1 H of CH_2(5′)). ^{13}C-NMR (126 MHz, $CDCl_3$): 158.7 (MMTr); 158.4 (MMTr); 154.3 (C(6)); 151.6 (C(2)); 147.9 (C(4)); 145.0, 143.9 (MMTr); 138.1 (C(8)); 137.1, 134.8, 130.4, 130.2, 128.9, 128.2, 127.9, 127.0 (MMTr); 121.2 (C(5)); 113.2 (MMTr); 91.0 (C(1′)); 86.8 (C(4′)); 86.5 (MMTr); 76.1 (C(2′)); 72.9 (C(3′)); 71.1 (MMTr); 63.7 (C(5′)); 55.2 (MeO of MMTr). HR-ESI-MS: 812.3420 ($C_{50}H_{46}N_5O_6^+$; calc. 812.3443).

5′-O,N^6-*Bis(4-methoxytrityl)-3′*-O-*(pivaloyloxymethyl)adenosine* (= *3′*-O-*{[(2,2-Dimethylpropanoyl)-oxy]methyl}*-N-*[(4-methoxyphenyl)(diphenyl)methyl]-5′*-O-*[(4-methoxyphenyl)(diphenyl)methyl]adenosine*; **10**). Compound **9** (0.60 mmol, 0.49 g), dried over P_2O_5 overnight, was dissolved in dry THF (5 ml), and 1 equiv. of NaH (24 mg of 60% dispersion, 0.60 mmol) was added. After stirring for 1 h at r.t., the mixture was added into a mixture of (pivaloyloxy)methyl chloride (0.66 mmol, 94 µl) and NaI (9 mg). The reaction was allowed to proceed for 5 h and quenched by adding H_2O. The mixture was extracted three times with Et_2O, and the Et_2O layer was dried (Na_2SO_4) and evaporated to dryness. The products were separated by SiO_2 chromatography AcOEt/petroleum ether (PE) 1:1 (*v*/*v*). Three products were obtained. According to ^{1}H-NMR data, in addition to the desired product **10** (0.12 g, 21% yield), its 2′-*O*-isomer (0.04 g) and, unexpectedly, 5′-*O*,N^6-bis(4-methoxytrityl)-3′-*O*-pivaloyladenosine (0.20 g) were formed. ^{1}H-NMR (500 MHz, $CDCl_3$): 8.02 (*s*, H–C(2)); 8.01 (*s*, H–C(8)); 7.22–7.38 (*m*, 24 H of MMTr); 7.02 (*s*, NH); 6.80–6.84 (*m*, 4 H of MMTr); 5.93 (*d*, *J* = 6.5, H–C(1′)); 5.43 (*d*, *J* = 6.5, 1 H of OCH_2O); 5.39 (*d*, *J* = 6.5, 1 H of OCH_2O); 4.93 (*dd*, *J* = 6.5, 5.5, H–C(2′)); 4.74 (*d*, *J* = 5.5, HO–C(2′)); 4.51 (*dd*, *J* = 5.5, 3.0, H–C(3′)); 4.37–4.38 (*m*, H–C(4′)); 3.80 (*s*, 2 MeO of MMTr); 3.48 (*dd*, *J* = 10.5, 4.0, 1 H of CH_2(5′)); 3.28 (*dd*, *J* = 10.5, 4.0, 1 H of CH_2(5′)); 1.17 (*s*, Me_3C of Piv). ^{13}C-NMR (126 MHz, $CDCl_3$): 177.8 (CO of Piv); 158.7 (C(6)); 158.3 (MMTr); 152.0 (C(2)); 148.3 (C(4)); 145.13 (MMTr); 138.6 (C(8)); 126.9, 127.9, 128.2, 128.9, 130.2, 130.3, 134.9 (MMTr); 121.3 (C(5)); 113.2 (MMTr); 89.7 (C(1′)); 88.7 (OCH_2O); 86.8 (MMTr); 83.6 (C(4′)); 79.3 (C(3′)); 74.7 (C(2′)); 71.1 (MMTr); 63.2 (C(5′)); 55.2 (MeO of MMTr); 38.7 (Me_3C of Piv); 27.0 (Me of Piv). HR-ESI-MS: 926.4115 ($C_{56}H_{56}N_5O_8^+$; calc. 926.4123).

2′-O-*Levulinoyl-3′*-O-*(pivaloyloxymethyl)adenosine* (= *3′*-O-*{[(2,2-Dimethylpropanoyl)oxy]methyl}-2′*-O-*(4-oxopentanoyl)adenosine*; **11**). Lev_2O was prepared by dissolving levulinic acid (5.6 mmol, 0.65 g) in dry 1,4-dioxane (10 ml) on an ice bath and adding dicyclohexylcarbodiimide (DCC; 2.8 mmol, 0.58 g) into the soln. in small portions within 1 h. The ice bath was removed, and the reaction was allowed to proceed at r.t. for 2 h. Precipitated dicyclohexylurea was filtered off and washed with 5 ml of dry dioxane. The filtrate was added to a soln. of **10** (2.3 mmol, 2.1 g, dried over P_2O_5 overnight) in dry pyridine (9 ml), and a cat. amount of 4-(dimethylamino)pyridine (DMAP) was added. After stirring overnight at r.t., the mixture was evaporated to dryness. The residue was dissolved in CH_2Cl_2, washed

with sat. aq. $NaHCO_3$ and sat. aq. NaCl, and dried (Na_2SO_4). The org. phase was evaporated to dryness and co-evaporated with toluene. The compound was subjected to detritylation without purification. Accordingly, the crude product was dissolved in 80% (*v*/*v*) aq. AcOH (50 ml), and after stirring for 5 h at 40°, the mixture was evaporated to dryness. The residue was dissolved in CH_2Cl_2 and washed three times with H_2O. The org. phase was dried (Na_2SO_4) and evaporated to dryness. The product was purified by SiO_2 chromatography, eluting with CH_2Cl_2 containing 5% MeOH, to yield **11** (0.91 g, 84%). White foam. ^{1}H-NMR (500 MHz, $CDCl_3$): 8.33 (*s*, H–C(2)); 7.83 (*s*, H–C(8)); 6.54 (*m*, HO–C(5′)); 6.01 (*d*, *J* = 7.5, H–C(1′)); 5.74 (*dd*, *J* = 7.5, 5.5, H–C(2′)); 5.64 (*m*, NH); 5.54 (*d*, *J* = 6.5, 1 H of OCH_2O); 5.12 (*d*, *J* = 6.5, 1 H of OCH_2O); 4.80 (*m*, H–C(3′)); 4.35 (*m*, H–C(4′)); 3.98 (*m*, 1 H of CH_2(5′)); 3.77 (*m*, 1 H of CH_2(5′)); 2.49–2.75 (*m*, CH_2CH_2 of Lev); 2.16 (*s*, Me of Lev); 1.23 (*s*, Me_3C of Piv). ^{13}C-NMR (126 MHz, $CDCl_3$): 206.1 (CO of Lev); 177.8 (CO of Piv); 171.5 (CO of Lev); 155.9 (C(6)); 152.7 (C(2)); 148.7 (C(4)); 140.6 (C(8)); 121.3 (C(5)); 88.9 (OCH_2O); 88.9 (C(1′)); 87.3 (C(4′)); 78.3 (C(3′)); 74.3 (C(2′)); 62.7 (C(5′)); 38.9 (Me_3C of Piv); 37.7 (CH_2 of Lev); 29.8 (Me of Lev); 27.5 (CH_2 of Lev); 27.0 (Me of Piv). HR-ESI-MS: 480.2072 ($C_{21}H_{30}N_5O_8^+$; calc. 480.2089).

*5′-*O*-[(*tert*-Butyl)dimethylsilyl]-2′-*O*-levulinoyl-N⁶-(4-methoxytrityl)-3′-*O*-(pivaloyloxymethyl)adenosine* (= *5′*-O-*[(*tert*-Butyl)(dimethyl)silyl]-3′*-O-*{[(2,2-dimethylpropanoyl)oxy]methyl}*-N⁶-*[(4-methoxyphenyl)(diphenyl)methyl]-2′*-O-*(4-oxopentanoyl)adenosine*; **12**). Compound **11** (1.7 mmol, 0.82 g) was co-evaporated twice from dry pyridine and dissolved in the same solvent (5 ml). ^{t}BuMe$_2$SiCl (2.1 mmol; 0.31 g) was added, and the mixture was stirred overnight at r.t. The reaction was quenched with MeOH, and the mixture was evaporated to dryness. The residue was dissolved in CH_2Cl_2 and washed with sat. aq. $NaHCO_3$ and sat. aq. NaCl. The org. phase was dried (Na_2SO_4) and evaporated to dryness. The product was purified by SiO_2 chromatography, eluting with CH_2Cl_2 containing 10% MeOH, and subjected directly to tritylation. Accordingly, the product (1.6 mmol, 0.94 g) was co-evaporated twice from dry pyridine and dissolved in dry pyridine (6 ml). MMTrCl (1.9 mmol, 0.59 g) was added, and the mixture was stirred over two nights at 40°. The reaction was quenched with MeOH, and the mixture was evaporated to dryness. The residue was dissolved in CH_2Cl_2 and washed with H_2O and sat. aq. NaCl. The org. phase was dried (Na_2SO_4) and evaporated to dryness. The product was purified by SiO_2 chromatography, eluting with CH_2Cl_2 containing 1–3% MeOH, to yield **12** (1.27 g, 85%). Yellowish foam. ^{1}H-NMR (500 MHz, $CDCl_3$): 8.06 (*s*, H–C(2)); 8.03 (*s*, H–C(8)); 7.20–7.35 (*m*, 12 H of MMTr); 6.89 (*s*, NH); 6.79 (*m*, 2 H of MMTr); 6.18 (*d*, *J* = 5.0, H–C(1′)); 5.68 (*m*, H–C(2′)); 5.37 (*d*, *J* = 6.5, 1 H of OCH_2O); 5.18 (*d*, *J* = 6.5, 1 H of OCH_2O); 4.78 (*m*, H–C(3′)); 4.20 (*dd*, *J* = 7.5, 3.0, H–C(4′)); 3.92 (*dd*, *J* = 11.3, 3.3, 1 H of CH_2(5′)); 3.80 (*dd*, *J* = 11.3, 3.3, 1 H of CH_2(5′)); 3.78 (*s*, MeO of MMTr); 2.56–2.75 (*m*, CH_2CH_2 of Lev); 2.14 (*s*, Me of Lev); 1.21 (*s*, Me_3C of Piv); 0.89 (*s*, Me_3CSi); 0.07 (*s*, MeSi); 0.05 (*s*, MeSi). ^{13}C-NMR (126 MHz, $CDCl_3$): 206.1 (CO of Lev); 177.8 (CO of Piv); 171.7 (CO of Lev); 158.3 (MMTr); 154.1 (C(6)); 152.5 (C(2)); 148.8 (C(4)); 145.2 (MMTr); 138.5 (C(8)); 137.2, 130.2, 128.9, 127.9, 126.9 (MMTr); 121.2 (C(5)); 113.2 (MMTr); 88.3 (OCH_2O); 85.9 (C(1′)); 83.9 (C(4′)); 76.2 (C(3′)); 74.9 (C(2′)); 71.0 (MMTr); 62.6 (C(5′)); 55.2 (MeO of MMTr); 38.8 (Me_3C of Piv); 37.8 (CH_2 of Lev); 29.7 (Me of Lev); 27.7 (CH_2 of Lev); 27.0 (Me of Piv); 25.9 (Me_3CSi); 18.4 (Me_3CSi); −5.4, −5.5 (MeSi). HR-ESI-MS: 866.4145 ($C_{47}H_{60}N_5O_9Si^+$; calc. 866.4155).

*5′-*O*-[(*tert*-Butyl)dimethylsilyl]-*N⁶*-(4-methoxytrityl)-3′-*O*-(pivaloyloxymethyl)adenosine* (= *5′*-O-*[(*tert*-Butyl)(dimethyl)silyl]-3′*-O-*{[(2,2-dimethylpropanoyl)oxy]methyl}*-N⁶-*[(4-methoxyphenyl)(diphenyl)methyl]adenosine*; **2b**). Compound **12** was dissolved in a soln. of $NH_2NH_2 \cdot H_2O$ (6.0 mmol, 0.29 ml) in pyridine (10 ml) and AcOH (2 ml) on an ice bath, and the mixture was stirred for 1.5 h. The ice bath was removed, and the reaction was allowed to proceed at r.t. for 5 h. The reaction was quenched with sat. aq. $NaHCO_3$, and the mixture was extracted with CH_2Cl_2. The org. phase was washed with sat. aq. NaCl and dried (Na_2SO_4) and evaporated to dryness. The product was purified by SiO_2 chromatography, eluting with CH_2Cl_2 containing 3% MeOH, to give **2b** (1.04 g, 92%). Yellowish foam. ^{1}H-NMR (500 MHz, $CDCl_3$): 8.03 (*s*, H–C(2)); 8.01 (*s*, H–C(8)); 7.23–7.37 (*m*, 12 H of MMTr); 6.97 (*s*, NH); 6.81 (*d*, *J* = 9, 2 H of MMTr); 5.95 (*d*, *J* = 5.5, H–C(1′)); 5.44–5.49 (*m*, OCH_2O); 4.75 (*m*, H–C(2′)); 4.51 (*dd*, *J* = 5.3, 2.8, H–C(3′)); 4.41 (*d*, *J* = 5.0, OH); 4.30 (*m*, H–C(4′)); 3.88 (*dd*, *J* = 11.3, 3.8, 1 H of CH_2(5′)); 3.80–3.83 (*m*, H–C(5″), MeO of MMTr); 1.25 (*s*, Me_3C of Piv); 0.86 (*s*, Me_3CSi); 0.08 (*s*, MeSi); 0.04 (*s*, MeSi). ^{13}C-NMR (126 MHz, $CDCl_3$): 177.9 (CO of Piv); 158.3 (MMTr); 154.2 (C(6)); 152.1 (C(2)); 148.3 (C(4)); 145.2 (MMTr); 138.4 (C(8)); 137.2, 130.2, 128.9, 127.9, 126.9 (MMTr); 121.2 (C(5)); 113.2

(MMTr); 89.3 (C(1′)); 88.5 (OCH_2O); 84.4 (C(4′)); 79.0 (C(3′)); 74.9 (C(2′)); 71.0 (MMTr); 62.8 (C(5′)); 55.2 (MeO of MMTr); 38.7 (Me_3C of Piv); 27.0 (Me of Piv); 25.8 (Me_3CSi); 18.3 (Me_3CSi); −5.5 (MeSi).

2′-O,5′-O,N⁶-Tris(4-methoxytrityl)adenosine (= *N⁶-[(4-Methoxyphenyl)(diphenyl)methyl]-2′,5′-bis-O-[(4-methoxyphenyl)(diphenyl)methyl]adenosine*; **13**). Adenosine (18.7 mmol, 5.00 g) was dried on P_2O_5 overnight. The nucleoside was co-evaporated from dry pyridine and dissolved in the same solvent (50 ml). MMTrCl (59.9 mmol, 18.5 g) was added, and the mixture was stirred at 60° overnight. The reaction was quenched by adding MeOH (50 ml), and the volatiles were removed under reduced pressure. The residue was dissolved in CH_2Cl_2, washed with H_2O and brine, and the org. layer was dried (Na_2SO_4) and evaporated to dryness. The residue was purified in three portions by SiO_2 chromatography, using CH_2Cl_2 containing 0–2% MeOH as eluent, to give **13** was (10.3 g, 50%). ^{1}H-NMR (500 MHz, $CDCl_3$): 7.98 (*s*, H–C(2)); 7.97 (*s*, H–C(8)); 7.00–7.43 (*m*, 36 H of MMTr); 6.84, 6.73, 6.66 (3*d*, *J* = 8.5, 2 H of MMTr); 6.38 (*d*, *J* = 7.5, H–C(1′)); 5.09 (*dd*, *J* = 7.5, 4.5, H–C(2′)); 4.08 (*dd*, *J* = 3.0, 3.5, H–C(4′)); 3.77, 3.78, 3.79 (3*s*, 3 H of MMTr); 3.31 (*dd*, *J* = 11.0, 3.5, 1 H of CH_2(5′)); 3.00 (*dd*, *J* = 11.0, 3.0, 1 H of CH_2(5′)); 2.92 (*d*, *J* = 4.5, H–C(3′)); 2.32 (*s*, OH). ^{13}C-NMR (126 MHz, $CDCl_3$): 159.2, 158.6, 158.3 (MMTr); 154.1 (C(6)); 152.4 (C(2)); 149.4 (C(4)); 145.3, 144.4, 143.9, 143.8, 143.0 (MMTr); 139.1 (C(8)); 137.3, 135.2, 134.5, 130.4, 130.2, 130.2, 128.9, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.5, 127.4, 127.2, 127.0, 126.8 (MMTr); 121.2 (C(5)); 113.5, 113.1, 113.1 (MMTr); 87.5 (C(1′)); 86.8, 86.1 (MMTr); 84.3 (C(4′)); 77.0 (C(2′), under $CDCl_3$); 71.0 (MMTr); 70.6 (C(3′)); 63.8 (C(5′)); 55.2 (MeO of MMTr). HR-ESI-MS: 1084.4649 ($C_{70}H_{62}N_5O_7^+$; calc. 1084.4644).

2′-O,5′-O,N⁶-Tris(4-methoxytrityl)-3′-O-[(methylsulfanyl)methyl]adenosine (= *N⁶-[(4-Methoxyphenyl)(diphenyl)methyl]-2′,5′-bis-O-[(4-methoxyphenyl)(diphenyl)methyl]-3′-O-[(methylsulfanyl)methyl]-adenosine*; **14**). Compound **13** (1.72 mmol, 1.86 g) was dried by co-evaporation from dry pyridine and twice from dry MeCN. The residue was dissolved in dry DMF (4.0 ml), and the soln. was cooled to 0° on an ice-bath. NaH (3.4 mmol, 0.137 g of 60% dispersion in oil) and NaI (1.5 mmol, 0.230 g) were added. The mixture was stirred for 0.5 h in an ice-bath, after which $MeSCH_2Cl$ (2.1 mmol, 170 µl) was added, and the stirring was continued for 4 h at r.t. Another portion of $MeSCH_2Cl$ (1.0 mmol, 85 µl) was added, and the stirring was continued for 0.5 h. The reaction was quenched by H_2O. The mixture was extracted three times with Et_2O. The combined org. phase was washed with brine, dried (Na_2SO_4), and evaporated to dryness. SiO_2 chromatography with 30–40% AcOEt in PE gave **14** (0.86 g, 43%) yield. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.77 (*s*, H–C(2)); 7.70 (*s*, H–C(8)); 6.95–7.43 (*m*, 36 H of MMTr); 6.85 (*s*, NH); 6.83, 6.77, 6.64 (3*d*, *J* = 9.0, 2 H of MMTr); 6.05 (*d*, *J* = 6.5, H–C(1′)); 5.33 (*dd*, *J* = 6.5, 5.0, H–C(2′)); 4.58 (*d*, *J* = 11.5, 1 H of OCH_2S); 4.20 (*m*, H–C(4′)); 4.18 (*d*, *J* = 11.5, 1 H of OCH_2S); 3.71, 3.78, 3.81 (3*s*, 3 H of MMTr); 3.57 (*dd*, *J* = 4.5, 2.0, H–C(3′)); 3.37 (*dd*, *J* = 10.5, 5.5, 1 H of CH_2(5′)); 3.17 (*dd*, *J* = 10.5, 4.5, 1 H of CH_2(5′)); 2.08 (*s*, MeS). HR-ESI-MS: 1144.4692 ($C_{72}H_{66}N_5O_7S^+$; calc. 1144.4677).

3′-O-[(Methylsulfanyl)methyl]adenosine (**15**). Nucleoside **14** (2.15 mmol, 2.46 g) was dissolved in 80% AcOH, the mixture was stirred overnight at r.t., evaporated to dryness, and co-evaporated twice from H_2O. The product was purified by SiO_2 chromatography, eluting with 10–20% MeOH in CH_2Cl_2, to yield **15** (0.49 g, 70%). ^{1}H-NMR (500 MHz, CD_3OD): 8.33 (*s*, H–C(2)); 8.21 (*s*, H–C(8)); 5.98 (*d*, *J* = 6.5, H–C(1′)); 4.92 (*d*, *J* = 12.0, 1 H of OCH_2S); 4.87–4.89 (*m*, 1 H of OCH_2S, H–C(2′)); 4.52 (*dd*, *J* = 5.5, 2.5, H–C(3′)); 4.29 (*m*, H–C(4′)); 3.91 (*dd*, *J* = 12.5, 2.5, 1 H of CH_2(5′)); 3.78 (*dd*, *J* = 12.5, 2.5, 1 H of CH_2(5′)); 2.22 (*s*, MeS). ^{13}C-NMR (126 MHz, CD_3OD): 156.1 (C(6)); 152.2 (C(2)); 149.0 (C(4)); 140.5 (C(8)); 121.2 (C(5)); 89.8 (C(1′)); 84.9 (C(4′)); 75.6 (C(3′)); 74.6 (OCH_2S); 73.6 (C(2′)); 61.9 (C(5′)); 12.4 (MeS). HR-ESI-MS: 328.1067 ($C_{12}H_{18}N_5O_4S^+$; calc. 328.1074).

*5′-O-[(*tert*-Butyl)dimethylsilyl]-3′-O-[(methylsulfanyl)methyl]-N⁶-(4-methoxytrityl)adenosine* (= *5′-O-[(*tert*-Butyl)(dimethyl)silyl]-N⁶-[(4-methoxyphenyl)(diphenyl)methyl]-3′-O-[(methylsulfanyl)methyl]-adenosine*; **16**). Nucleoside **15** (2.5 mmol, 0.81 g) dried on P_2O_5 overnight was dissolved in dry pyridine (9.0 ml). ^{t}BuMe$_2$SiCl (3.0 mmol, 0.45 g) was added, and the mixture was stirred overnight at r.t. The reaction was quenched with MeOH, and the mixture was evaporated to dryness. The product was purified by eluting through a thin layer of SiO_2 with 10% MeOH in CH_2Cl_2. The volatiles were removed under reduced pressure, and the residue was dried by coevaporating twice with anh. pyridine and dissolved in the same solvent (10 ml). MMTrCl (2.7 mmol, 0.84 g) was added, and the mixture was stirred overnight at 40°. The reaction was quenched with MeOH, and the mixture was evaporated to dryness. The residue was dissolved in CH_2Cl_2 and washed with H_2O, sat. aq. $NaHCO_3$, and sat. aq. NaCl. The org.

layer was dried (Na_2SO_4) and evaporated to dryness. The product was purified by SiO_2 chromatography, eluting with 1–2% MeOH in CH_2Cl_2, to give **16** (1.56 g, 88%). Yellowish foam. ^{1}H-NMR (500 MHz, $CDCl_3$): 8.05 (*s*, H–C(2)); 8.04 (*s*, H–C(8)); 7.23–7.38 (*m*, 12 H of MMTr); 6.97 (*s*, NH); 6.81 (*d*, *J* = 7.0, 2 H of MMTr); 5.99 (*d*, *J* = 5.5, H–C(1′)); 4.88 (*d*, *J* = 11.5, 1 H of OCH_2S); 4.83 (*d*, *J* = 11.5, 1 H of OCH_2S); 4.71 (*m*, H–C(2′)); 4.52 (*dd*, *J* = 5.0, 3.0, H–C(3′)); 4.37 (*d*, *J* = 5.5, OH); 4.31 (*m*, H–C(4′)); 3.89 (*dd*, *J* = 11.5, 4.0, 1 H of CH_2(5′)); 3.83 (*dd*, *J* = 11.5, 3.0, 1 H of CH_2(5′)); 3.80 (*s*, 3 H of MMTr); 2.23 (*s*, MeS), 0.87 (*s*, Me_3CSi); 0.10 (*s*, MeSi); 0.06 (*s*, MeSi). ^{13}C-NMR (126 MHz, $CDCl_3$): 158.3 (MMTr); 154.2 (C(6)); 152.0 (C(2)); 148.4 (C(4)); 145.2 (MMTr); 138.2 (C(8)); 137.2, 130.2, 128.9, 127.9, 126.9 (MMTr); 121.2 (C(5)); 113.2 (MMTr); 89.5 (C(1′)); 84.3 (C(4′)); 76.2 (C(3′)); 75.3 (OCH_2S); 75.0 (C(2′)); 71.0 (MMTr); 62.8 (C(5′)); 55.2 (MeO of MMTr); 25.9 (*Me*$_3$CSi); 18.3 (Me_3*C*Si); 14.2 (MeS), −5.5, −5.6 (MeSi). HR-ESI-MS: 714.3140 ($C_{38}H_{48}N_5O_5SSi^+$; calc. 714.3140).

5′-O-*[(*tert*-Butyl)dimethylsilyl]-2′*-O-*levulinoyl*-N^6-*(4-methoxytrityl)-3′*-O-*[(methylsulfanyl)methyl]-adenosine* (= *5′*-O-*[(*tert*-Butyl)(dimethyl)silyl]*-N^6-*[(4-methoxyphenyl)(diphenyl)methyl]-3′*-O-*[(methylsulfanyl)methyl]-2′*-O-*(4-oxopentanoyl)adenosine*; **17**). Levulinic acid (5.5 mmol, 0.63 g) was dissolved in dry dioxane (10.0 ml). The mixture was stirred on an ice-bath, DCC (2.7 mmol, 0.56 g) was added portionwise within 1 h, and stirring was continued at r.t. for 2 h. Dicyclohexylurea was filtered off and washed with dioxane (5.0 ml). The washings were combined to the filtrate, and **16** (2.2 mmol, 1.56 g; dried on P_2O_5) in dry pyridine (9.0 ml) was added. A cat. amount of DMAP was added, and the mixture was stirred overnight at r.t. The mixture was evaporated to dryness, the residue was dissolved in CH_2Cl_2, washed with sat. aq. $NaHCO_3$ and sat. aq. NaCl, and the org. phase was dried (Na_2SO_4). The product was purified by SiO_2 chromatography, eluting with 1% MeOH in CH_2Cl_2, to yield **17** (1.57 g, 88%). ^{1}H-NMR (500 MHz, $CDCl_3$): 8.11 (*s*, H–C(2)); 8.06 (*s*, H–C(8)); 7.23–7.37 (*m*, 12 H of MMTr); 6.94 (*s*, NH); 6.81 (*d*, *J* = 9.0, 2 H of MMTr); 6.23 (*d*, *J* = 4.5, H–C(1′)); 5.69 (*dd*, *J* = 4.5, 5.0, H–C(2′)); 4.79 (*dd*, *J* = 5.0, 5.5, H–C(3′)); 4.75 (*d*, *J* = 11.5, 1 H of OCH_2S); 4.60 (*d*, *J* = 11.5, 1 H of OCH_2S); 4.22 (*m*, H–C(4′)); 4.01 (*dd*, *J* = 11.5, 2.5, 1 H of CH_2(5′)); 3.86 (*dd*, *J* = 11.5, 2.5, 1 H of CH_2(5′)); 3.80 (*s*, 3 H of MMTr); 2.61–2.80 (*m*, 4 H of Lev); 2.18 (*s*, MeS); 2.17 (*s*, 3 H of Lev); 0.94 (*s*, Me_3CSi); 0.13 (*s*, MeSi); 0.11 (*s*, MeSi). HR-ESI-MS: 812.3474 ($C_{43}H_{54}N_5O_7SSi^+$; calc. 812.3508).

3′-O-*(Acetyloxymethyl)-5′*-O-*[(*tert*-butyl)dimethylsilyl]-2′*-O-*levulinoyl*-N^6-*(4-methoxytrityl)adenosine* (= *3′*-O-*[(Acetyloxy)methyl]-5′*-O-*[(*tert*-butyl)(dimethyl)silyl]*-N^6-*[(4-methoxyphenyl)(diphenyl)-methyl]-2′*-O-*(4-oxopentanoyl)adenosine*; **18**). Nucleoside **17** (1.04 mmol, 0.84 g) was dried on P_2O_5 overnight and dissolved in dry CH_2Cl_2 (7.0 ml) under N_2. SO_2Cl_2 in CH_2Cl_2 (1.24 mmol, 1.14 ml of 1 mol l^{-1} soln.) was added dropwise, and the mixture was stirred for 1 h at r.t. The volatiles were removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (3.0 ml) and added dropwise to a mixture of AcOK (1.78 mmol, 0.175 g) and dibenzo-18-crown-6 (0.77 mol, 0.28 g) in dry CH_2Cl_2. After 2 h stirring at r.t., the mixture was diluted with AcOEt and washed with H_2O and brine. The org. phase was dried (Na_2SO_4). The mixture was concentrated under reduced pressure, and the crown ether precipitate was removed by filtration. The product was purified on a SiO_2 column, eluting with CH_2Cl_2 containing 1% MeOH, to give **18** (0.78 g, 91%). ^{1}H-NMR (500 MHz, $CDCl_3$): 8.06 (*s*, H–C(2)); 8.04 (*s*, H–C(8)); 7.23–7.37 (*m*, 12 H of MMTr); 6.93 (*s*, NH); 6.81 (*d*, *J* = 9.0, 2 H of MMTr); 6.19 (*d*, *J* = 4.5, H–C(1′)); 5.71 (*dd*, *J* = 4.5, 5.0, H–C(2′)); 5.30 (*d*, *J* = 6.5, 1 H of OCH_2O); 5.27 (*d*, *J* = 6.5, 1 H of OCH_2O); 4.80 (*dd*, *J* = 5.0, 5.5, H–C(3′)); 4.21 (*m*, H–C(4′)); 3.97 (*dd*, *J* = 11.5, 3.0, 1 H of CH_2(5′)); 3.83 (*dd*, *J* = 11.5, 3.0, 1 H of CH_2(5′)); 3.81 (*s*, 3 H of MMTr); 2.61–2.78 (*m*, 4 H of Lev); 2.17 (*s*, 3 H of Lev); 2.11 (*s*, Me of Ac); 0.92 (*s*, Me_3CSi); 0.11 (*s*, MeSi); 0.09 (*s*, MeSi). ^{13}C-NMR (126 MHz, $CDCl_3$): 206.1 (CO of Lev); 171.7 (CO of Lev); 170.4 (CO of Ac); 158.3 (MMTr); 154.1 (C(6)); 152.5 (C(2)); 148.6 (C(4)); 145.2 (MMTr); 138.4 (C(8)); 137.2 (MMTr); 130.2 (MMTr); 128.9 (MMTr); 127.9 (MMTr); 126.9 (MMTr); 121.2 (C(5)); 113.2 (MMTr); 88.3 (OCH_2O); 86.2 (C(1′)); 83.5 (C(4′)); 76.2 (C(3′)); 74.8 (C(2′)); 71.0 (MMTr); 62.1 (C(5′)); 55.2 (MMTr); 37.8 (Lev); 29.8 (Lev); 27.7 (Lev); 25.9 (TBDMS); 21.0 (*Me*COOCH$_2$); 18.4 (TBDMS); −5.5 (TBDMS); −5.4 (TBDMS). HR-ESI-MS: 824.3641 ($C_{44}H_{54}N_5O_9Si^+$; calc. 824.3685).

3′-O-*[(Acetyloxy)methyl]-5′*-O-*[(*tert*-butyl)dimethylsilyl]*-N^6-*(4-methoxytrityl)adenosine* (= *3′*-O-*[(Acetyloxy)methyl]-5′*-O-*[(*tert*-butyl)(dimethyl)silyl]*-N^6-*[(4-methoxyphenyl)(diphenyl)methyl]adenosine*; **2a**). Nucleoside **17** (0.78 mmol, 0.64 g) was dissolved in dry CH_2Cl_2 (18 ml). NH_2NH_3OAc (1.17 mmol; 0.107 g) in dry MeOH (2.0 ml) was added, and the mixture was stirred at r.t. for 1 h. The reaction was quenched with 1.5 equiv. of acetone, and the mixture was stirred for 15 min and evaporated

to dryness. The product was purified on a SiO_2 column, eluting with CH_2Cl_2 containing 1–2% MeOH, to afford **2a** (0.44 g, 78%). ^{1}H-NMR (500 MHz, $CDCl_3$): 8.03 (*s*, H–C(2)); 8.01 (*s*, H–C(8)); 7.23–7.37 (*m*, 12 H of MMTr); 6.96 (*s*, NH); 6.81 (*d*, *J* = 9.0, 2 H of MMTr); 5.94 (*d*, *J* = 6.0, H–C(1′)); 5.46 (*d*, *J* = 6.5, 1 H of OCH_2O); 5.44 (*d*, *J* = 6.5, 1 H of OCH_2O); 4.71 (*m*, H–C(2′)); 4.51 (*m*, H–C(3′)); 4.46 (*d*, *J* = 5.5, OH); 4.32 (*m*, H–C(4′)); 3.87 (*dd*, *J* = 11.5, 4.0, 1 H of CH_2(5′)); 3.81–3.83 (*m*, 3 H of MMTr, H–C(5″)); 3.81 (*s*, 3 H of MMTr); 2.14 (*s*, Me of Ac); 0.85 (*s*, Me_3CSi); 0.08 (*s*, MeSi); 0.03 (*s*, MeSi). ^{13}C-NMR (126 MHz, $CDCl_3$): 170.5 (CO); 158.3 (MMTr); 154.2 (C(6)); 152.0 (C(2)); 148.0 (C(4)); 145.1 (MMTr); 138.3 (C(8)); 137.2, 130.2, 128.9, 127.9, 126.9 (MMTr); 121.2 (C(5)); 113.2 (MMTr); 89.5 (C(1′)); 88.5 (OCH_2O); 84.6 (C(4′)); 79.3 (C(3′)); 75.2 (C(2′)); 71.0 (MMTr); 62.8 (C(5′)); 55.2 (MeO of MMTr); 25.8 (Me_3CSi); 21.1 (Ac); 18.2 (Me_3CSi); −5.5, −5.6 (MeSi). HR-ESI-MS: 726.3294 ($C_{39}H_{48}N_5O_7Si^+$; calc. 726.3318).

2′,3′-Di-O-*levulinoyladenosine* (= *2′,3′-Bis*-O-*(4-oxopentanoyl)adenosine*; **19**). Lev_2O was prepared by dissolving levulinic acid (29.6 mmol, 3.43 g) in dry 1,4-dioxane (40 ml) on an ice-bath and adding DCC (14.8 mmol, 3.05 g) into the soln. in small portions within 1 h. The ice-bath was removed, and the reaction was allowed to proceed at r.t. for 2 h. Precipitated dicyclohexylurea was filtered off, and the precipitate was washed with 10 ml of dry dioxane. The filtrate was added to a soln. of **9** (7.4 mmol, 6.00 g) in dry pyridine (30 ml), and a cat. amount of DMAP was added. After 2 h at r.t., the mixture was evaporated to dryness. The residue was dissolved in CH_2Cl_2, and washed with sat. aq. $NaHCO_3$ and sat. aq. NaCl. The org. phase was dried (Na_2SO_4) and evaporated to dryness. The compound was subjected to detritylation without purification. The crude product was dissolved in 80% (*v*/*v*) aq. AcOH (80 ml). After stirring overnight at r.t., the mixture was evaporated to dryness. The product was purified by SiO_2 chromatography, eluting with CH_2Cl_2 containing 5–10% MeOH, to give **19** (2.79 g, 81%). White foam. 81% yield (2.79 g). ^{1}H-NMR (500 MHz, $CDCl_3$): 8.34 (*s*, H–C(2)); 7.88 (*s*, H–C(8)); 6.71 (br. *d*, *J* = 10.0, OH); 6.06 (*d*, *J* = 7.5, H–C(1′)); 6.01 (*dd*, *J* = 7.5, 5.0, H–C(2′)); 5.73 (*dd*, *J* = 5.0, 1.0, H–C(3′)); 4.38–4.39 (*m*, H–C(4′)); 4.00 (*dd*, *J* = 13.5, 1.5, 1 H of CH_2(5′)); 3.85 (*m*, 1 H of CH_2(5′)); 2.67–2.84 (*m*, 3 CH_2 of Lev); 2.54–2.56 (*m*, CH_2 of Lev); 2.25 (*s*, Me of Lev); 2.17 (*s*, Me of Lev). ^{13}C-NMR (126 MHz, $CDCl_3$): 206.4 (OC=O); 206.3 (OC=O); 171.8 (CC=O); 171.2 (CC=O); 156.0 (C(6)); 152.6 (C(2)); 148.6 (C(4)); 140.5 (C(8)); 121.1 (C(5)); 88.8 (C(1′)); 86.6 (C(4′)); 73.0 (C(2′)); 72.9 (C(3′)); 62.7 (C(5′)); 37.8 (CH_2C=O of Lev); 37.7 (CH_2C=O of Lev); 29.9 (Me of Lev); 29.8 (Me of Lev); 27.7 (CH_2C=OO of Lev); 27.4 (CH_2C=OO of Lev). HR-ESI-MS: 464.1797 ($C_{20}H_{26}N_5O_8^+$; calc. 464.1776).

2′,3′-Di-O-*levulinoyl*-N^6-*(4-methoxytrityl)adenosine* (= N-*[(4-Methoxyphenyl)(diphenyl)methyl]-2′,3′-bis*-O-*(4-oxopentanoyl)adenosine*; **4**). Compound **19** (3.2 mmol, 1.5 g) was evaporated twice from dry pyridine and dissolved in the same solvent (25 ml). Me_3SiCl (8.1 mmol, 1.03 ml) was added, and the mixture was stirred for 1.5 h. Another portion of Me_3SiCl (8.1 mmol, 1.03 ml) was added, and stirring was continued for 1 h. MMTrCl (3.6 mmol, 1.1 g) was added, and the mixture was stirred overnight at 35°. Since the reaction was not completed according to TLC analysis, the mixture was stirred at 45° for 7 h. Then, MMTrCl (0.7 mmol, 0.2 g) was added, and the mixture was stirred at 40° overnight. Sat. aq. $NaHCO_3$ was added, and the mixture was stirred for 10 min and extracted with AcOEt. The org. phase was washed with sat. aq. $NaHCO_3$, dried (Na_2SO_4) and evaporated to dryness. The product was purified by SiO_2 chromatography, eluting with CH_2Cl_2 containing 3% MeOH. The products were not separated, and the purification was repeated, eluting with AcOEt containing 5% MeOH. Two products were obtained: **4** (0.67 g) and its 5′-*O*-trimethylsilyl derivative (0.96 g). The Me_3Si group was removed from the latter by treatment with Bu_4NF in THF under acidic conditions. Accordingly, Bu_4NF (2.5 mmol, 0.65 g) was dissolved in dry THF (16 ml), and AcOH (3 ml) was added. The nucleoside was added, and the mixture was stirred at r.t. for 15 min. Sat. aq. $NaHCO_3$ soln. was added, and the mixture was extracted with CH_2Cl_2. The org. phase was washed with sat. aq. NaCl soln., dried (Na_2SO_4), and evaporated to dryness to yield **4**. Yellowish foam. ^{1}H-NMR (500 MHz, $CDCl_3$): 8.03 (*s*, H–C(2)); 7.83 (*s*, H–C(8)); 7.23–7.36 (*m*, 12 H of MMTr); 7.04 (*s*, NH); 6.83–6.84 (*m*, 2 H of MMTr, OH); 6.06 (*d*, *J* = 8.0, H–C(1′)); 5.94 (*dd*, *J* = 8.0, 5.5, H–C(2′)); 5.71 (*dd*, *J* = 5.5, 0.5, H–C(3′)); 4.36–4.37 (*m*, H–C(4′)); 3.95 (*dd*, *J* = 13.0, 1.0, 1 H of CH_2(5′)); 3.79–3.83 (*m*, MeO of MMTr, H–C(5″)); 2.53–2.80 (*m*, 4 CH_2 of Lev); 2.24 (*s*, Me of Lev); 2.19 (*s*, Me of Lev). ^{13}C-NMR (126 MHz, $CDCl_3$): 206.4 (OC=O); 206.2 (OC=O); 171.7 (CC=O); 171.2 (CC=O); 158.3 (MMTr); 154.6 (C(6)); 151.9 (C(2)); 147.2 (C(4)); 144.9 (MMTr); 139.8 (C(8)); 136.8 (MMTr); 130.1, 128.8, 127.9, 126.9 (MMTr); 122.4 (C(5)); 113.2 (MMTr); 88.7 (C(1′)); 86.6

(C(4′)); 73.1 (C(2′)); 72.9 (C(3′)); 71.1 (MMTr); 62.6 (C(5′)); 55.2 (MeO of MMTr); 37.7 (*C*H_2C=O of Lev); 37.6 (*C*H_2C=O of Lev); 29.8 (Me of Lev); 29.8 (Me of Lev); 27.6 (*C*H_2C=OO of Lev); 27.4 (*C*H_2C=OO of Lev). HR-ESI-MS: 736.2944 ($C_{40}H_{42}N_5O_9^+$; calc. 736.2977).

*3′-*O*-(Acetyloxymethyl)-5′-*O*-[(*tert*-butyl)dimethylsilyl]-*N^6*-(4-methoxytrityl)adenosin-2′-yl 2′,3′-Di-*O*-levylinoyl-*N^6*-(4-methoxytrityl)adenosin-5′-yl 3-Acetyloxy-2,2-bis(ethoxycarbonyl) Phosphate* (= *Diethyl ({[[[(2*R*,3*R*,4*R*,5*R*)-4-[(Acetyloxy)methoxy]-5-({[(*tert*-butyl)(dimethyl)silyl]oxy}methyl)-2-(6-{[(4-methoxyphenyl)(diphenyl)methyl]amino}-9*H*-purin-9-yl)tetrahydrofuran-3-yl]oxy}({(2*R*,3*R*,4*R*,5*R*)-5-(6-{[(4-methoxyphenyl)(diphenyl)methyl]amino}-9*H*-purin-9-yl)-3,4-bis[(4-oxopentanoyl)oxy]tetrahydrofuran-2-yl}methoxy)phosphoryl]oxy}methyl)[(acetyloxy)methyl]propanedioate*; **7a**). Nucleoside **2a** (0.69 mmol, 0.500 g) was dried on P_2O_5 overnight and dissolved in dry CH_2Cl_2 (3 ml) under N_2. Et_3N (3.45 mmol; 0.479 ml) and $(Et_2N)_2PCl$ (0.90 mmol, 0.188 ml) were added, and the mixture was stirred under N_2 for 2 h. The product was isolated by passing the mixture through a short SiO_2 column with AcOEt/hexane containing 0.5% Et_3N 7:3. The solvent was removed under reduced pressure, and the residue was co-evaporated from dry MeCN and dry CH_2Cl_2 to remove the traces of Et_3N. The identitiy of the product (*3′-*O*-[(acetyloxy)methyl]-2′-*O*-[bis(diethylamino)phosphanyl]-5′-*O*-[(*tert*-butyl)(dimethyl)silyl]-*N^6*-[(4-methoxyphenyl)(diphenyl)methyl]adenosine*; **3a**) was checked by ^{31}P- and ^{1}H-NMR spectroscopy. ^{1}H-NMR (500 MHz, CD_3CN): 8.14 (*s*, H–C(2)); 7.88 (*s*, H–C(8)); 7.25–7.41 (*m*, 12 H of MMTr); 6.93 (*s*, NH); 6.86 (*d*, *J* = 9.0, 2 H of MMTr); 6.05 (*d*, *J* = 6.5, H–C(1′)); 5.41 (*d*, *J* = 6.5, 1 H of OCH_2O); 5.35 (*d*, *J* = 6.5, 1 H of OCH_2O); 4.96 (*ddd*, *J* = 11.0, 6.0, 4.5, H–C(2′)); 4.51 (*dd*, *J* = 4.5, 2.5, H–C(3′)); 4.12 (*m*, H–C(4′)); 3.97 (*dd*, *J* = 11.5, 4.5, 1 H of CH_2(5′)); 3.86 (*dd*, *J* = 11.5, 3.5, 1 H of CH_2(5′)); 3.78 (*s*, 3 H of MMTr); 2.85–2.97 (*m*, 2 CH_2 of EtN); 2.65–2.72 (*m*, 2 CH_2 of NEt); 2.17 (*s*, Me of Ac); 1.00 (*t*, *J* = 7.0, 2 Me of EtN); 0.95 (*s*, 3 Me of TBDMS); 0.77 (*t*, *J* = 7.5, 2 Me of NEt); 0.12 (*s*, Me of TBDMS); 0.11 (*s*, Me of TBDMS). ^{31}P-NMR (202 MHz, CD_3CN): 137.6.

Compound **3a** was dissolved in dry MeCN (1.0 ml) under N_2. 1*H*-Tetrazole (0.68 mmol, 1.51 ml of 0.45 mol l^{-1} soln. in MeCN) and **4** (0.48 mmol, 0.355 g) in MeCN (1.0 ml) were added. The reaction was allowed to proceed for 25 min. After this period, 1*H*-tetrazole (0.78 mmol, 1.74 ml of 0.45 mol l^{-1} soln. in MeCN) and diethyl *2-(acetyloxymethyl)-2-(hydroxymethyl)malonate* (**5**; 0.69 mmol, 0.180 g) were added. The course of the reaction was monitored by ^{31}P-NMR spectroscopy. After 1 h, ^{31}P-NMR signals (202 MHz, CD_3CN) at 140.7 and 140.5 ppm were observed. The phosphite ester (*diethyl ({[[[(2*R*,3*R*,4*R*,5*R*)-4-[(acetyloxy)methoxy]-5-({[(*tert*-butyl)(dimethyl)silyl]oxy}methyl)-2-(6-{[(4-methoxyphenyl)(diphenyl)methyl]amino}-9*H*-purin-9-yl)tetrahydrofuran-3-yl]oxy}({(2*R*,3*R*,4*R*,5*R*)-5-(6-{[(4-methoxyphenyl)(diphenyl)methyl]amino}-9*H*-purin-9-yl)-3,4-bis[(4-oxopentanoyl)oxy]tetrahydrofuran-2-yl}methoxy)phosphanyl]oxy}methyl)[(acetyloxy)methyl]propanedioate*; **6a**) formed was oxidized with I_2 (0.2 g) in a mixture of THF (4.0 ml), H_2O (2.0 ml), and 2,6-lutidine (1.0 ml). The oxidation was allowed to proceed overnight. The excess of I_2 was destroyed with 5% $NaHSO_3$. The mixture was extracted twice with CH_2Cl_2. The org. phase was washed with brine, dried on Na_2SO_4 and evaporated to dryness. The crude product was purified on a SiO_2 column eluting with a 4:1 mixture of AcOEt and CH_2Cl_2. The overall yield of **7a** starting from **2a** was 40% (0.49 g). ^{1}H-NMR (500 MHz, $CDCl_3$): 8.12, 8.10, 8.08, 8.06, 8.04, 8.01, 8.00, 7.98 (8*s*, 2 H–C(2), 2 H–C(8)); 7.22–7.38 (*m*, 24 H of MMTr); 6.95, 6.94, 6.92, 6.91 (4*s*, 2 NH); 6.79–6.83 (*m*, 4 H of MMTr); 6.21 (*d*, *J* = 1.5, 0.5 H of H–C(1′)); 6.19 (*d*, *J* = 5.5, 0.5 H of H–C(1′)); 6.19 (*d*, *J* = 2.0, 0.5 H of H–C(1′)); 6.12 (*d*, *J* = 5.5, 0.5 H of H–C(1′)); 5.83 (*dd*, *J* = 5.5, 5.5, 0.5 H of H–C(2′)); 5.80 (*dd*, *J* = 5.5, 5.5, 0.5 H of H–C(2′)); 5.70 (*m*, H–C(3′)); 5.50 (*m*, H–C(2′)); 5.44 (*d*, *J* = 6.5, 0.5 H of OC*H*$_2$OAc); 5.43 (*d*, *J* = 6.5, 0.5 H of OC*H*$_2$OAc); 5.39 (*m*, H–C(3′)); 5.36 (*d*, *J* = 6.5, 0.5 H of OC*H*$_2$OAc); 5.28 (*d*, *J* = 6.5, 0.5 H of OC*H*$_2$OAc); 4.57–4.69 (*m*, POCH_2C, C*H*$_2$OAc); 4.33–4.43 (*m*, H–C(4′), H–C(5′), H–C(5″)); 4.12–4.25 (*m*, H–C(4′), 2 MeC*H*$_2$O); 4.00 (*m*, 1 H of CH_2(5′)); 3.83 (*m*, 1 H of CH_2(5′)); 3.80, 3.80, 3.80, 3.79 (4*s*, 2 MeO of MMTr); 2.56–2.83 (*m*, 4 CH_2 of Lev); 2.21, 2.17, 2.15, 2.13 (4*s*, 2 Me of Lev); 2.10, 2.08, 2.00, 1.92 (4*s*, 2 Me of Ac); 1.15–1.25 (*m*, 2 *Me*CH_2); 0.88, 0.87 (2*s*, Me_3CSi); 0.08, 0.06, 0.03, 0.01 (4*s*, 2 MeSi). ^{31}P-NMR (202 MHz, CD_3CN): −2.4 and −2.5. (Multiplicity of some signals is due to the presence of (R_P)- and (S_P)-diastereoisomers.) HR-ESI-MS: 1768.6720 ($C_{90}H_{105}N_{10}O_{24}PSi^+$; calc. 1768.6805).

*3-Acetyloxy-2,2-bis(ethoxycarbonyl) 3′-*O*-(Acetyloxymethyl)-N^6-(4-methoxytrityl)adenosin-2′-yl 2′,3′-Di-*O*-levylinoyl-N^6-(4-methoxytrityl)adenosin-5′-yl Phosphate* (= *Diethyl ({[[[(2*R*,3*R*,4*R*,5*R*)-4-[(Acetyloxy)methoxy]-5-(hydroxymethyl)-2-(6-{[(4-methoxyphenyl)(diphenyl)methyl]amino}-9*H*-pu-*

*rin-9-yl)tetrahydrofuran-3-yl]oxy}[(2*R*,3*R*,4*R*,5*R*)-5-(6-{[(4-methoxyphenyl)(diphenyl)methyl]amino}-9*H*-purin-9-yl)-3,4-bis[(4-oxopentanoyl)oxy]tetrahydrofuran-2-yl}methoxy)phosphoryl]oxy}methyl)[(acetyloxy)methyl]propanedioate*; **8a**). Compound **7a** (0.27 mmol, 0.47 g), dried over P_2O_5 overnight, was dissolved in dry THF (5 ml). $Et_3N \cdot 3$ HF (1.06 mmol, 173 µl) was added, and the mixture was stirred for 3 d at r.t. The mixture was neutralized by adding aq. Et_3NHOAc (2.0 mol l^{-1}) in small portions. The mixture was evaporated to dryness, and the residue was then dissolved in CH_2Cl_2 and washed with H_2O. The org. phase was evaporated to dryness. The product was purified by SiO_2 chromatography, eluting with 3% MeOH in CH_2Cl_2, to give **8a** (0.41 g, 93%). ^{1}H-NMR (500 MHz, $CDCl_3$): 8.07, 8.04, 8.03, 8.02, 8.00, 7.98, 7.96, 7.95 (8*s*, 2 H–C(2), 2 H–C(8)); 7.19–7.35 (*m*, 24 H of MMTr); 7.04 (br. *d*, *J* = 3.0, NH); 6.94 (br. *s*, NH); 6.76–6.81 (*m*, 4 H of MMTr); 6.45 (*m*, HO–C(5′)); 6.17 (*d*, *J* = 5.5, 0.5 H of H–C(1′)); 6.14 (*d*, *J* = 5.5, 0.5 H of H–C(1′)); 6.06 (*d*, *J* = 7.0, 0.5 H of H–C(1′)); 5.82 (*dd*, *J* = 5.5, 5.5, 0.5 H of H–C(2′)); 5.77 (*dd*, *J* = 5.5, 5.5, 0.5 H of H–C(2′)); 5.71 (*m*, 0.5 H of H–C(2′)); 5.66 (*m*, 0.5 H of H–C(2′)); 5.54 (*d*, *J* = 6.8, 0.5 H of OC*H*$_2$OAc); 5.53 (*m*, 0.5 H of H–C(3′)); 5.43 (*m*, 0.5 H of H–C(3′)); 5.36 (*s*, 1 H of OC*H*$_2$OAc); 5.24 (*d*, *J* = 6.5, 0.5 H of OC*H*$_2$OAc); 4.77 (*dd*, *J* = 5.0, 1.5, 0.5 H of H–C(3′)); 4.68 (*dd*, *J* = 5.0, 1.5, 0.5 H of H–C(3′)); 4.61 (*s*, 0.5 H of C*H*$_2$OAc); 4.02–4.53 (*m*, 1.5 H of C*H*$_2$OAc, POC*H*$_2$C, 2 H–C(4′), 1.5 CH$_2$(5′), 1.5 CH$_2$(5″)); 3.86–3.95 (*m*, 1 H of CH$_2$(5′)); 3.75–3.79 (*m*, 2 MeO of MMTr); 3.65–3.72 (*m*, 1 H of CH$_2$(5″)); 2.51–2.82 (*m*, 4 CH$_2$ of Lev); 2.19, 2.18, 2.13, 2.12 (4*s*, Me of Lev); 2.08, 2.06, 1.98, 1.95 (4*s*, 2 Me of Ac); 1.13–1.21 (*m*, 2 *Me*CH$_2$). ^{13}C-NMR (126 MHz, $CDCl_3$): 206.3, 206.3, 206.1, 206.1 (CO of Lev); 171.8, 171.7, 171.6, 171.5 (CO of Lev); 170.6, 170.4, 170.1, 170.0 (CO of Ac); 166.3, 166.2 (*C*=OOEt), 158.4, 158.3 (MMTr); 154.6, 154.2 (C(6)); 152.6, 151.7 (C(2)); 148.7, 147.3 (C(4)); 145.1, 145.1, 144.9 (MMTr); 140.5, 138.8 (C(8)); 137.2, 137.0, 130.2, 128.9, 128.8, 128.0, 127.9, 127.9, 127.0, 126.9, 126.9 (MMTr); 122.5, 121.3 (C(5)); 113.2, 113.2, 113.2 (MMTr); 89.3 (C(1′)); 88.9, 88.2 (OCH$_2$O); 86.5, 86.2 (C(4′)); 85.9 (C(1′)); 80.7, 80.1 (C(4′)); 78.8, 78.3, 77.1–77.3 (C3′, under $CDCl_3$), 73.3, 73.0 (C(2′)); 71.1, 71.1, 71.0 (MMTr); 70.5, 70.4 (C(2′)); 67.5, 67.2 (C(5′)); 65.7 (PO*C*H$_2$C), 62.4 (C(5′)); 62.3 (Me*C*H$_2$); 62.0, 61.1 (*C*H$_2$OAc); 58.0 (–C–); 55.2 (MeO of MMTr); 37.7, 37.6 (*C*H$_2$C=O of Lev); 29.8, 29.7 (Me of Lev); 27.6, 27.5, 27.5 (*C*H$_2$C=OO Lev, 21.0, 21.0, 20.6, 20.6 (Ac); 13.9 (*Me*CH$_2$). ^{31}P-NMR (202 MHz, $CDCl_3$): −2.3, −2.7. (Multiplicity of some signals is due to the presence of (R_P)- and (S_P)-diastereoisomers.) HR-ESI-MS: 1675.5600 ($C_{84}H_{89}N_{10}NaO_{24}P^+$; calc. 1675.5687).

*3-Acetyloxy-2,2-bis(ethoxycarbonyl) 3′-*O*-(Acetyloxymethyl)-*N^6*-(4-methoxytrityl)adenosin-2′-yl 2′,3′-Di-*O*-levylinoyl-*N^6*-(4-methoxytrityl)adenosin-5′-yl Phosphate* (= *Diethyl {[({[(2*R*,3*R*,4*R*,5*R*)-4-[(Acetyloxy)methoxy]-2-(6-amino-9*H*-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3-yl]oxy}[(2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl]methoxy}phosphoryl)oxy]methyl}[(acetyloxy)methyl]propanedioate*; **1a**). Compound **8a** (0.35 mmol, 0.58 g) was evaporated from dry MeCN, and the residue was dissolved in dry CH_2Cl_2 (8 ml). NH_2NH_3OAc (1.17 mmol, 0.107 g) in dry MeOH (0.9 ml) was added, and the mixture was stirred at r.t. for 2.5 h. The reaction was quenched with acetone, and the mixture was stirred for 20 min and evaporated to dryness. The product was purified on a SiO_2 column eluting with CH_2Cl_2 containing 5% MeOH. The compound was then subjected to detritylation with 80% (*v*/*v*) aq. AcOH (10 ml). After stirring overnight at r.t., the mixture was evaporated to dryness, and the residue was co-evaporated twice with H_2O. The product was purified first by SiO_2 chromatography, eluting with CH_2Cl_2 containing 10–20% MeOH, and then by HPLC on a *Sun Fire™ Prep C18* column (250 × 10 mm, 5 µm; flow rate 3.0 ml min^{-1}; 150 × 4.6 mm, 5 µm; flow rate 1.0 ml min^{-1}), using a linear gradient elution from 33 to 100% MeOH in 20 min, to afford **1a** (153 mg, 39%). ^{1}H-NMR (500 MHz, CD_3CN): 7.99–8.25 (*m*, 2 H–C(2), 2 H–C(8)); 6.43, 6.34, 6.27, 6.18 (4 br. *s*, 2 NH, HO–C(5′)); 6.09 (*m*, H–C(1′)); 5.96 (*d*, *J* = 4.5, 0.5 H of H–C(1′)); 5.91 (br. *d*, *J* = 4.0, 0.5 H of H–C(1′)); 5.54 (*m*, H–C(2′)); 5.47 (*d*, *J* = 6.5, 0.5 H of OCH$_2$O); 5.40 (*d*, *J* = 7.0, 0.5 H of OCH$_2$O); 5.30 (*m*, 1 H of OCH$_2$O); 4.66 (*dd*, *J* = 5.0, 2.5, 0.5 H of H–C(3′)); 4.61–4.65 (*m*, 0.5 H of H–C(3′), 0.5 H of H–C(2′)); 4.54 (*m*, 0.5 H of H–C(2′)); 4.46–4.51 (*m*, 1 H of CH$_2$(5′), 1 H of CC*H*$_2$OAc); 4.38–4.44 (*m*, 1 H of CH$_2$(5″), 0.5 H of CC*H*$_2$OAc); 4.28–4.35 (*m*, 0.5 H of H–C(3′), 0.5 H of CC*H*$_2$OAc, 1 H of POCH$_2$C, H–C(4′)); 4.10–4.25 (*m*, 0.5 H of H–C(3′), 2 MeC*H*$_2$, 1 H of POCH$_2$C); 4.08 (*m*, 0.5 H of H–C(4′)); 4.03 (*m*, 0.5 H of H–C(4′)); 3.85 (*m*, 1 H of CH$_2$(5′)); 3.70 (*m*, 1 H of CH$_2$(5′)); 2.10 (*s*, 1.5 H of AcO); 2.07 (*s*, 1.5 H of AcO); 2.01, 1.97 (2*s*, Me of OCH$_2$O*Ac*), 1.13–1.23 (*m*, 2 *Me*CH$_2$). ^{13}C-NMR (126 MHz, CD_3CN): 170.4, 170.1 (CO of Ac); 166.4, 166.3 (*C*=OOEt), 156.5, 156.0 (C(6)); 152.9, 152.5 (C(2)); 148.6 (C(4)); 140.8, 140.6, 139.6, 139.3 (C(8)); 119.7, 120.6 (C(5)); 88.7 (C(1′)); 88.3 (OCH$_2$O); 87.99 (C(1′));

85.8, 85.7, 82.4, 82.0 (C(4′)); 78.4, 78.1 (C(3′)); 76.7, 76.5 (C(2′)); 74.0, 73.9 (C(2′)); 70.2, 70.0 (C(3′)); 67.8, 67.6 (PO*C*H_2C); 65.5, 65.4 (C(5′), PO*C*H_2C); 62.4, 62.3 (Me*C*H_2); 61.8, 61.7 (C(5′)); 60.9, 60.8 (*C*H_2OAc); 57.9 (–C–); 20.3 (Ac); 19.8 (OCH$_2$O*Ac*); 13.2 (*Me*CH_2). ^{31}P-NMR (202 MHz, CD_3CN): −2.5, −2.4. (Multiplicity of some signals is due to the presence of (R_P)- and (S_P)-diastereoisomers.) HR-ESI-MS: 913.2707 ($C_{34}H_{46}N_{10}O_{18}P^+$; calc. 913.2724).

3-(Acetyloxy)-2,2-bis(ethoxycarbonyl) 5′-O-*[(*tert-*Butyl)dimethylsilyl]*-N^6-*(4-methoxytrityl)-3′*-O-*(pivaloyloxymethyl)adenosin-2′-yl 2′,3′-Di*-O-*levylinoyl*-N^6-*(4-methoxytrityl)adenosin-5′-yl Phosphate* (= *Diethyl [(Acetyloxy)methyl]({[[[(2*R,*3*R,*4*R,*5*R*)-5-({[(*tert-*butyl)(dimethyl)silyl]oxy}methyl)-4-{[(2,2-dimethylpropanoyl)oxy]methoxy}-2-(6-{[(4-methoxyphenyl)(diphenyl)methyl]amino}-9*H*-purin-9-yl)tetrahydrofuran-3-yl]oxy}({(2*R,*3*R,*4*R,*5*R*)-5-(6-{[(4-methoxyphenyl)(diphenyl)methyl]amino}-9*H*-purin-9-yl)-3,4-bis[(4-oxopentanoyl)oxy]tetrahydrofuran-2-yl}methoxy)phosphoryl]oxy}methyl)propanedioate*; **7b**). Nucleoside **2b** (0.73 mmol, 0.600 g) was dried over P_2O_5 overnight and dissolved in dry CH_2Cl_2 (4 ml) under N_2. Et_3N (3.91 mmol, 0.543 ml) and $(Et_2N)_2PCl$ (0.94 mmol, 0.197 ml) were added, and the mixture was stirred under N_2 for 2 h. The product was isolated by passing the mixture through a short SiO_2 column with AcOEt/hexane containing 0.5% Et_3N 7:3. The solvent was removed under reduced pressure, and the residue was co-evaporated from dry MeCN and dry CH_2Cl_2 to remove the traces of Et_3N. The identitiy of the product (*2′*-O-*[bis(diethylamino)phosphanyl]-5′*-O-*[(*tert-*butyl)(dimethyl)silyl]-3′*-O-*{[(2,2-dimethylpropanoyl)oxy]methyl}*-N^6-*[(4-methoxyphenyl)(diphenyl)methyl]adenosine*; **3b**) was checked by ^{31}P- and ^{1}H-NMR spectroscopy. ^{1}H-NMR (500 MHz, CD_3CN): 8.13 (*s*, H–C(2)); 7.88 (*s*, H–C(8)); 7.24–7.41 (*m*, 12 H of MMTr); 6.92 (*s*, HN–C(6)); 6.83–6.87 (*m*, 2 H of MMTr); 6.05 (*d*, *J* = 6.5, H–C(1′)); 5.47 (*d*, *J* = 6.5, 1 H of OCH$_2$O); 5.34 (*d*, *J* = 6.5, 1 H of OCH$_2$O); 4.97 (*m*, H–C(2′)); 4.50 (*dd*, *J* = 4.8, 2.8, H–C(3′)); 4.23 (*m*, H–C(4′)); 3.98 (*dd*, *J* = 11.5, 4.5, 1 H of CH$_2$(5′)); 3.84 (*dd*, *J* = 11.5, 3.5, 1 H of CH$_2$(5′)); 3.78 (*s*, 3 H of MMTr); 2.84–2.98 (*m*, 4 H of EtN); 2.62–2.70 (*m*, 4 H of EtN); 1.22 (*s*, Me_3C); 1.00 (*t*, *J* = 7.5, 6 H of EtN); 0.94 (*s*, Me_3CSi); 0.75 (*t*, *J* = 7.5, 6 H of EtN); 0.11 (*s*, 2 MeSi). ^{31}P-NMR (202 MHz, CD_3CN): 137.2.

Compound **3b** was dissolved in dry MeCN (1.0 ml) under N_2. 1*H*-Tetrazole (0.73 mmol, 1.630 ml of 0.45 M soln. in MeCN) and **4** (0.55 mmol; 0.400 g) in dry MeCN (1.0 ml) were added. The reaction was allowed to proceed for 15 min. After this period, 1*H*-tetrazole (0.73 mmol, 1.630 ml of 0.45 M soln. in MeCN) and diethyl 2-(acetyloxymetyl)-2-(hydroxymethyl)malonate (0.86 mmol, 0.230 g) in dry MeCN were added. The course of the reaction was monitored by ^{31}P-NMR spectroscopy. After 40 min, ^{31}P-NMR signals (202 MHz, CD_3CN) at 140.6 and 140.5 ppm were observed.

The phosphite ester (*diethyl [(acetyloxy)methyl]({[[[(2*R,*3*R,*4*R,*5*R*)-5-({[(*tert-*butyl)(dimethyl)silyl]oxy}methyl)-4-{[(2,2-dimethylpropanoyl)oxy]methoxy}-2-(6-{[(4-methoxyphenyl)(diphenyl)methyl]amino}-9*H*-purin-9-yl)tetrahydrofuran-3-yl]oxy}({(2*R,*3*R,*4*R,*5*R*)-5-(6-{[(4-methoxyphenyl)(diphenyl)methyl]amino}-9*H*-purin-9-yl)-3,4-bis[(4-oxopentanoyl)oxy]tetrahydrofuran-2-yl}methoxy)phosphanyl]oxy}methyl)propanedioate*; **6b**) formed was oxidized with I_2 (0.2 g) in a mixture of THF (4.0 ml), H_2O (2.0 ml), and 2,6-lutidine (1.0 ml). The oxidation was allowed to proceed overnight. The oxidized product, **7b**, exhibited ^{31}P-NMR signals (202 MHz, CD_3CN) at −2.4 and −2.6 ppm. The excess of I_2 was destroyed with 5% $NaHSO_3$. The mixture was extracted three times with CH_2Cl_2. The org. phase was dried (Na_2SO_4) and evaporated to dryness. The crude product was purified by SiO_2 chromatography, eluting first with a 1:1 mixture of AcOEt and CH_2Cl_2, then with AcOEt, and finally with AcOEt containing 5% MeOH, to yield **7b** (0.50 g, 35%). ^{1}H-NMR (500 MHz, $CDCl_3$): 7.98–8.07 (*m*, 2 H–C(2), 2 H–C(8)); 7.21–7.38 (*m*, 24 H of MMTr); 6.94 (br. *s*, NH); 6.90 (br. *s*, NH); 6.78–6.82 (*m*, 4 H of MMTr); 6.19 (*d*, *J* = 2.0, H–C(1′)); 6.13 (*d*, *J* = 5.5, H–C(1′)); 5.78 (*dd*, *J* = 5.3, 5.3, H–C(2′)); 5.68 (*dd*, *J* = 5.3, 5.3, H–C(2′)); 5.58 (*m*, H–C(3′)); 5.52 (*d*, *J* = 6.3, 1 H of OCH$_2$O); 5.32 (*d*, *J* = 6.3, 1 H of OCH$_2$O); 5.03 (*m*, H–C(3′)); 4.54–4.65 (*m*, CC*H*$_2$OAc, POC*H*$_2$C); 4.17–4.38 (*m*, 2 H–C(4′), 1 H of CH$_2$(5′), 1 H of CH$_2$(5″), 2 MeC*H*$_2$); 3.94 (*m*, 1 H of CH$_2$(5′)); 3.78–3.83 (*m*, 1 H of CH$_2$(5″), 2 MeO of MMTr); 2.55–2.78 (*m*, 2 CH$_2$CH$_2$ of Lev); 2.18, 2.14 (2*s*, 2 Me of Lev); 2.01 (*s*, Me of Ac); 1.23 (2*s*, Me_3C of Piv); 1.15–1.25 (*m*, 2 *Me*CH$_2$); 0.83 (*s*, Me_3CSi); 0.03 (*s*, 2 MeSi). ^{31}P-NMR (202 MHz, $CDCl_3$): −2.4, −2.6. (Multiplicity of some signals is due to the presence of (R_P)- and (S_P)-diastereoisomers.)

3-(Acetyloxy)-2,2-bis(ethoxycarbonyl) 2′,3′-Di-O-*levylinoyl*-N^6-*(4-methoxytrityl)adenosin-5′-yl* N^6-*(4-Methoxytrityl)-3′*-O-*(pivaloyloxymethyl)adenosin-2′-yl Phosphate* (= *Diethyl [(Acetyloxy)methyl]({[[[(2*R,*3*R,*4*R,*5*R*)-4-{[(2,2-dimethylpropanoyl)oxy]methoxy}-5-(hydroxymethyl)-2-(6-{[(4-me-*

*thoxyphenyl)(diphenyl)methyl]amino}-9*H*-purin-9-yl)tetrahydrofuran-3-yl]oxy}({(2*R*,3*R*,4*R*,5*R*)-5-(6-{[(4-methoxyphenyl)(diphenyl)methyl]amino}-9*H*-purin-9-yl)-3,4-bis[(4-oxopentanoyl)oxy]tetrahydrofuran-2-yl}methoxy)phosphoryl]oxy}methyl)propanedioate*; **8b**). The ^{t}BuMe$_2$Si group was removed by treatment with Bu_4NF in THF under acidic conditions. Accordingly, Bu_4NF (0.8 mmol, 0.22 g) was dissolved in dry THF (6.8 ml), and AcOH (1.2 ml) was added. Compound **7b** (0.3 mmol, 0.50 g) was added, and the mixture was stirred at r.t. for 2 d. $NaHCO_3$ soln. (1%) was added, and the mixture was extracted twice with CH_2Cl_2. The org. phase was dried (Na_2SO_4) and evaporated to dryness. The product was purified by SiO_2 chromatography, eluting with CH_2Cl_2 containing 3–5% MeOH, to afford a diastereoisomeric mixture (1:1) of **8b** (0.33 g, 70%). Clear oil. ^{1}H-NMR (500 MHz, $CDCl_3$): 8.04, 8.03, 8.03, 8.01, 8.00, 7.98, 7.95, 7.95 (8*s*, 2 H–C(2), 2 H–C(8)); 7.19–7.35 (*m*, 24 H of MMTr); 7.04 (*m*, NH); 6.92 (br. *s*, NH); 6.77–6.81 (*m*, 4 H of MMTr); 6.48 (*dd*, *J* = 2.0, 11.5, 0.5 H of HO–C(5′)); 6.40 (*dd*, *J* = 2.5, 11.5, 0.5 H of HO–C(5′)); 6.16 (*d*, *J* = 5.5, 0.5 H of H–C(1′)); 6.14 (*d*, *J* = 5.0, 0.5 H of H–C(1′)); 6.04 (*d*, *J* = 6.5, 0.5 H of H–C(1′)); 6.03 (*d*, *J* = 7.0, 0.5 H of H–C(1′)); 5.80 (*t*, *J* = 5.5, 0.5 H of H–C(2′)); 5.77 (*t*, *J* = 5.5, 0.5 H of H–C(2′)); 5.71 (*m*, 0.5 H of H–C(3′)); 5.64–5.67 (*m*, 0.5 H of H–C(3′), 0.5 H of OCH_2O); 5.50–5.56 (*m*, 0.5 H of H–C(2′), 0.5 H of OCH_2O); 5.46 (*m*, 0.5 H of H–C(2′)); 5.29 (*d*, *J* = 6.5, 0.5 H of OCH_2O); 5.19 (*d*, *J* = 6.5, 0.5 H of OCH_2O); 4.75 (*dd*, *J* = 1.5, 5.5, 0.5 H of H–C(3′)); 4.70 (*dd*, *J* = 1.5, 5.5, 0.5 H of H–C(3′)); 4.50–4.55 (*m*, 1 H of CCH_2OAc, 1 H of POCH_2C); 4.08–4.46 (*m*, 1 H of OCH_2OAc, POCH_2C, 2 H–C(4′), H–C(5′), H–C(5″), 2 MeCH_2); 3.88 (*m*, 0.5 H of H–C(5′), 0.5 H of H–C(5″)); 3.75–3.78 (*m*, 2 MeO of MMTr); 3.65–3.72 (*m*, 0.5 H of H–C(5′), 0.5 H of H–C(5″)); 2.55–2.80 (*m*, 2 CH_2CH_2 of Lev); 2.18, 2.17, 2.13, 2.12 (4*s*, 2 Me of Lev); 1.99, 1.95 (2*s*, Me of AcO); 1.20, 1.19 (2*s*, Me_3C of Piv); 1.13–1.22 (*m*, 2 *Me*CH_2). ^{13}C-NMR (126 MHz, $CDCl_3$): 206.3, 206.3, 206.1, 206.1 (CO of Lev); 177.7, 177.9 (CO of Piv); 171.8, 171.7, 171.6, 171.5 (CO of Lev); 170.1, 170.0 (CO of Ac); 166.3, 166.2, 166.1, 166.0 (*C*=OOEt); 158.4, 158.3 (MMTr); 154.6, 154.2 (C(6)); 152.6, 151.7 (C(2)); 148.7, 147.3 (C(4)); 145.2, 145.1, 145.1, 145.0 (MMTr); 140.5, 140.3, 138.6 (C(8)); 137.2, 137.0, 130.2, 128.9, 127.9, 127.9, 126.9, 126.9 (MMTr); 122.5, 121.3 (C(5)); 113.2, 113.2 (MMTr); 89.3 (C(1′)); 89.1, 88.9 (OCH_2O); 86.8, 86.6 (C(4′)); 86.0, 85.9 (C(1′)); 80.7 (C(4′)); 78.8, 78.6 (C(3′)); 73.3 (C(2′)); 71.0 (MMTr); 67.2 (C(5′)); 65.4 (POCH_2C), 62.3 (*C*H_2CH_3), 61.2 (*C*H_2OAc), 58.0 (–C–); 55.2 (MeO of MMTr); 38.8 (Me_3C of Piv); 37.8, 37.7, 37.7 (*C*H_2C=O of Lev); 29.8, 29.8 (Me of Lev); 27.6, 27.5, 27.5 (*C*H_2C=OO of Lev); 27.0 (Me of Piv); 20.6, 20.6 (Ac); 13.9 (*Me*CH_2). ^{31}P-NMR (202 MHz, $CDCl_3$): −2.2, −2.7. (Multiplicity of some signals is due to the presence of (R_P)- and (S_P)-diastereoisomers.) HR-ESI-MS: 1717.6164 ($C_{87}H_{95}N_{10}NaO_{24}P^+$; calc. 1717.6151).

*3-(Acetyloxy)-2,2-bis(ethoxycarbonyl) adenosin-5′-yl 3′-*O*-(pivaloyloxymethyl)adenosin-2′-yl Phosphate* (= *Diethyl [(Acetyloxy)methyl][[({[(2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl]methoxy}[(2*R*,3*R*,4*R*,5*R*)-2-(6-amino-9*H*-purin-9-yl)-4-{[(2,2-dimethylpropanoyl)oxy]methoxy}-5-(hydroxymethyl)tetrahydrofuran-3-yl]oxy}phosphoryl)oxy]methyl}propanedioate*; **1b**). To remove the levulinoyl groups, **8b** (0.087 mmol, 0.100 g) was dissolved in a soln. of $NH_2NH_2 \cdot H_2O$ (2.50 mmol, 0.078 ml) in a mixture of pyridine (4 ml) and AcOH (1 ml) on an ice-bath, and the mixture was stirred for 1 h. The ice-bath was removed, and the reaction was allowed to proceed at r.t. for 3 h. The reaction was quenched with 0.1M NaH_2PO_3 soln. (25 ml), and the product was extracted into CH_2Cl_2. The org. phase was washed with H_2O, dried (Na_2SO_4), and evaporated to dryness. The product was purified by SiO_2 chromatography, eluting with CH_2Cl_2 containing 5% MeOH. The compound was then subjected to detritylation with 80% (*v*/*v*) aq. AcOH (10 ml). After stirring overnight at r.t., the mixture was evaporated to dryness. The residue was co-evaporated twice with H_2O. The product was purified first by SiO_2 chromatography, eluting with CH_2Cl_2 containing 10–20% MeOH, and then by HPLC on a *Thermo Hypersil Hypurity™ Elite C18* column (150 × 4.6 mm, 5 μm, flow rate 1.0 ml min^{-1}), using a linear gradient elution from H_2O to MeCN in 30 min, to give **1b** (24 mg, 29%). ^{1}H-NMR (500 MHz, CD_3CN): 8.23, 8.20, 8.20, 8.11 (4*s*, 2 H–C(2)); 8.11, 8.06, 8.06, 8.00 (4*s*, 2 H–C(8)); 6.49, 6.38, 6.33, 6.22 (4 br. *s*, 2 NH, HO–C(5′)); 6.10 (*m*, H–C(1′)); 5.96 (*d*, *J* = 4.5, 0.5 H of H–C(1′)); 5.91 (br. *d*, *J* = 4.0, 0.5 H of H–C(1′)); 5.52–5.60 (*m*, H–C(2′), 0.5 H of OCH_2O); 5.50 (*d*, *J* = 7.0, 0.5 H of OCH_2O); 5.27 (*m*, 1 H of OCH_2O); 4.70 (*dd*, *J* = 5.0, 2.5, 0.5 H of H–C(3′)); 4.67 (*dd*, *J* = 5.0, 2.0, 0.5 H of H–C(3′)); 4.63 (*m*, 0.5 H of H–C(2′)); 4.53 (*m*, 0.5 H of H–C(2′)); 4.42–4.52 (*m*, H–C(5′), 1 H of CCH_2OAc); 4.30–4.42 (*m*, 1 H of OCH_2OAc, 0.5 H of H–C(3′), POCH_2C); 4.29 (*m*, H–C(4′)); 4.07–4.25 (*m*, 0.5 H of H–C(3′), H–C(5″), 2 MeCH_2, 0.5 H of H–C(4′)); 4.03 (*m*, 0.5 H of H–C(4′)); 3.85 (*m*, 1 H of CH_2(5′)); 3.69 (*m*,

1 H of CH_2(5′)); 1.96 (*s*, Me of AcO); 1.23, 1.21 (2*s*, Me_3C of Piv); 1.13–1.23 (*m*, 2 *Me*CH_2). ^{13}C-NMR (126 MHz, CD_3CN): 177.6, 177.6 (CO of Piv); 170.1, 170.0 (CO of Ac); 166.4, 166.3 (*C*=OOEt); 156.5, 156.0 (C(6)); 152.9, 152.5 (C(2)); 149.7, 148.6 (C(4)); 140.8, 140.5, 139.8, 139.1 (C(8)); 119.7, 120.6 (C(5)); 88.8 (OCH_2O); 88.8, 88.0, 88.0 (C(1′)); 85.9, 85.7, 82.4, 82.3 (C(4′)); 78.3, 78.0 (C(3′)); 76.7, 76.5, 74.0, 73.9 (C(2′)); 70.2, 70.0 (C(3′)); 67.8, 65.5 (C(5′)); 65.4, 65.3 (PO*C*H_2C), 62.4, 62.3 (Me*C*H_2); 61.8, 61.7 (C(5′)); 60.9, 60.8 (*C*H_2OAc), 57.9, 57.8 (–C–); 38.5, 38.5 (Me_3C of Piv); 26.2, 26.2 (Me of Piv); 19.8, 19.8 (Ac); 13.2, 13.2 (*Me*CH_2). ^{31}P-NMR (202 MHz, CD_3CN): −2.4, −2.4. (Multiplicity of some signals is due to the presence of (R_P)- and (S_P)-diastereoisomers.) HR-ESI-MS: 955.3215 ($C_{37}H_{52}N_{10}O_{18}P^+$; calc. 955.3193).

Kinetic Measurements. The reactions were carried out in sealed tubes immersed in a thermostated H_2O bath (37.0 ± 0.1°). The hydroxonium ion concentration of the reaction solns. (3.0 ml) was adjusted with 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (*HEPES*) buffer (0.036/0.024 mol l^{-1}; pH 7.5). The ionic strength of the solns. was adjusted to 0.1M with NaCl. The hydroxonium ion concentration of the buffer solns. was calculated with the aid of the known pK_a values of the buffer acids under the experimental conditions. The initial substrate concentration was *ca.* 0.15 mmol l^{-1}. The AcO group was removed with hog liver carboxyesterase (HLE; 2.6 U ml^{-1}). The samples (200 µl) withdrawn at appropriate intervals were made acidic (pH 2) with 1 M aq. HCl to inactivate the enzyme and to quench the hydrolysis, cooled in an ice-bath and filtered with minisart *RC 4* filters (0.20 µm). The reaction products were identified by the mass spectra (LC/MS) using a mixture of H_2O, MeCN, and HCOOH (0.1%) as an eluent (*Gemini C18* column (2 × 150 mm 5 µm; flow rate 200 µl min^{-1}). The composition of the samples was analyzed on a *Thermo ODS Hypersil C18* column (4 × 250 mm, 5 µm; flow rate 0.95 ml min^{-1}), using AcOH/AcONa buffer (0.045/0.015 mol l^{-1}) and MeCN, containing ammonium chloride (0.1M). A good separation of the product mixtures was obtained on using a 5 min isocratic elution with the buffer containing 2% MeCN, followed by a linear gradient (45 min) up to 43% MeCN. Signals were recorded on a UV detector at a wavelength of 260 nm. The enzymatic deacetylations obeyed first-order kinetics at the HLE concentrations employed.

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