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Synthesis of Nucleoside Di- and Triphosphates and Dinucleoside Polyphosphates with *cyclo*Sal-Nucleotides

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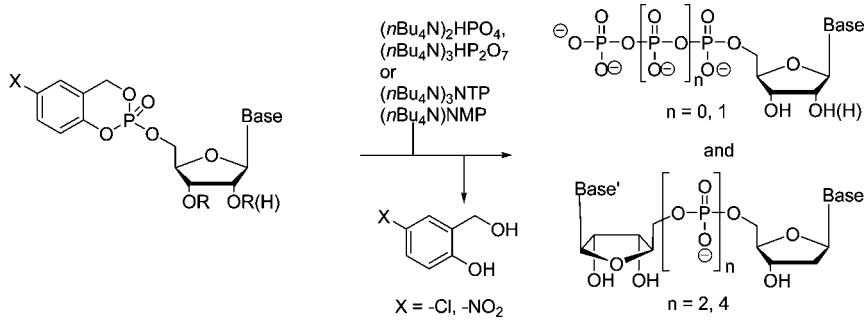
Received November 4, 2008



A new and efficient method for the synthesis of nucleoside di- and triphosphates as well as dinucleoside polyphosphates (Np_nN') is described. 5-Acceptor-substituted (5-nitro and 5-chloro) *cyclo*Sal-nucleotides are used as starting material that were reacted with a variety of phosphate nucleophiles as pyrophosphate or nucleotides to the corresponding products in short times and very good yields. After consumption of the starting *cyclo*Sal-phosphate triester, first the protecting groups were cleaved and finally the products were isolated after RP-column chromatography. Examples are shown for all five pyrimidine and purine bases found in natural nucleosides as well as one non-natural pyrimidine base to prove that the method can be applied generally.

Introduction

Nucleoside polyphosphates **1** like nucleoside triphosphates (NTPs) play essential roles in biological systems. In addition to the well-known function of ATP as the primary energy source in many systems, naturally occurring deoxyribo- and ribonucleoside triphosphates are fundamental building blocks for enzymatic DNA and RNA synthesis in vivo and in vitro. Modified nucleoside triphosphates, e.g., AZTTP or ddTTP, are important therapeutic antivirals and diagnostic agents that are needed for studies of numerous biochemical and pharmacological processes. Dinucleoside polyphosphates (Np_nN) **2** have been proposed as signaling and regulatory molecules for many different biological functions in most forms of life.[1] Two examples of possible application of the latter compounds are the inhibition of platelet aggregation[2] and the regulation of vasoactivity.[3]

A general and efficient method for the synthesis of these compounds and their analogues would facilitate studies of their possible medical applications. The synthesis of nucleoside diphosphates (NDPs) or NTPs as well as Np_nNs can be accomplished by both enzymatic and chemical methods.[4] A widely used chemical approach for the synthesis of these compounds is based on P^V-reagents and implies the coupling of a phosphate, a pyrophosphate anion, or a nucleoside triphosphate with activated nucleoside monophosphates such as phosphoramidates, phosphorimidazolidates, and phosphormorpholidates.[5−8] In most cases, long reaction times and tedious purification are required, and the yields are generally moderate to poor.

An alternative approach for the synthesis of NTPs based on P^{III}-chemistry was introduced by Ludwig and Eckstein.[9] Activated nucleoside phosphite triesters are converted with pyrophosphate into a cyclic intermediate and are subsequently oxidized and hydrolyzed to give NTPs in isolated yields up to 75%.[9] However, when we used this method for the synthesis of thymidine-5′-triphosphate (TTP), the yield obtained was less

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10.1021/jo802348h CCC: $40.75
Published on Web 03/25/2009

than 20%. Therefore, the method reported here is at least a complementary method for the efficient synthesis of NTPs. A modified Eckstein procedure has also been used for the synthesis of Np_nNs.[10]

Nevertheless, none of the methods mentioned above offers an universal access to the different target structures but only for one class of compounds.

We have reported on a new synthetic approach for the synthesis of nucleoside diphosphate pyranoses (NDP-sugars) employing *cyclo*Sal-nucleotides (*cyclo*Saligenyl-nucleotides) **3**. There, *cyclo*Sal-triesters were reacted in a fast and efficient way with pyranosyl-1-phosphates.[11] This method gave the sugar nucleotides in about 2-fold higher yields (up to 59%) compared to other literature-known chemical or chemoenzymatic methods.[12]

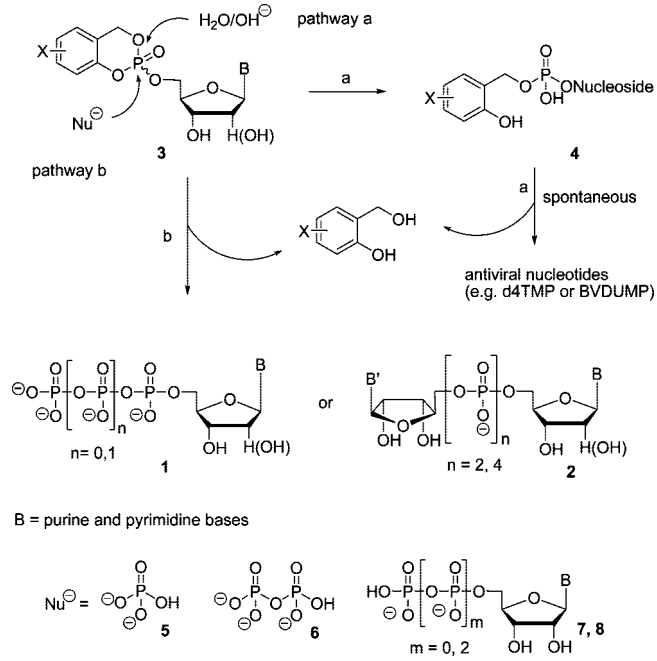
The *cyclo*Sal-technique has been developed previously as a prodrug concept to deliver biologically active nucleotides into cells.[13] The purely pH-dependent cleavage is initiated by nucleophilic attack of water/hydroxide on the neutral phosphate triester leading to an intermediate benzyl phosphate diester **4**. Subsequently, diester **4** is spontaneously cleaved to yield a nucleotide, e.g., the antivirals 2′,3′-dideoxy-2′,3′-didehydrothymidine monophosphate (d4TMP)[14] or 5-[(*E*)-bromovinyl]-2′-deoxyuridine monophosphate (BVDUMP)[15,16] and a salicyl alcohol (Scheme 1, pathway a). The technique has been applied successfully to a variety of nucleoside analogues and thus providing superior antiviral activity.[13−17]

Here, we report on the successful and highly efficient synthesis of nucleoside di- and triphosphates as well as dinucleoside polyphosphates using *cyclo*Sal activated nucleotides of six purine and pyrimidine bases.[18] Phosphate or pyrophosphate salts **5**, **6**, UMP **7**, and ATP **8** were used as nucleophiles to react with the *N*,*O*-protected *cyclo*Sal-nucleotides (see compounds **9** and **10**, Scheme 2) to give the corresponding (di)nucleoside polyphosphates **1** and **2** (Scheme 1, pathway b).

Results and Discussion

As starting material various *cyclo*Sal-nucleotides were used. To prove the general applicability of the method, all naturally occurring nucleosides (thymidine, uridine, adenosine, guanosine, and cytidine) as well as analogues of thymidine (*carba*-deoxythymidine (*carba*-dT)) and uridine (5-(*E*)-bromovinyl-2′-deoxyuridine (BVDU)) were used as the nucleosidic part.

SCHEME 1. Cleavage Mechanism of *cyclo*Sal-triesters



Generally, 5-nitro-substituted *cyclo*Sal-nucleotides **9a**−**e** were prepared because the electron-withdrawing effect of the nitro substituent results in sufficient electrophilicity at the phosphorus atom to allow a rapid reaction with the corresponding nucleophiles. Only in the case of uridine and *carba*-dT were the 5-chloro-substituted *cyclo*Sal-nucleotides **10a**,**b** used.

3′-*O*Ac-thymidine **11a**, N^6-Ac-2′,3′-*O*Ac-adenosine **11b**, N^2-formamidine-2′,3′-OAc-guanosine **11c**, N^4-Ac-2′,3′-*O*Ac-cytidine **11d**, 3′-*O*Ac-BVDU **11e**, 2′,3′-*O*-cyclopentyluridine **11f**, and 3′-*O*Ac-*carba*-thymidine **11g** have been synthesized according to literature procedures.[19−21] Conversion of the protected nucleosides **11** into the active phosphotriesters **9**,**10** was achieved by reaction with *cyclo*Sal-phosphochloridites **12a**,**b** and subsequent oxidation using oxone in 80−90% yield.[13,22]

Phosphochloridites **12a**,**b** were prepared in 70−80% yield from the corresponding salicyl alcohols **13a**,**b** with phosphorus trichloride (Scheme 2), while alcohols **13a**,**b** were prepared by reduction of 5-NO_2-salicylic aldehyde[23] and 5-Cl-salicylic acid,[24] respectively. All 5-nitro-substituted *cyclo*Sal-triesters **9a**−**e** were too sensitive to moisture to be purified by chromatography on a silica gel column or on a Chromatotron. The purity of the crude material of triesters **9a**−**e** obtained after the oxone oxidation and extraction was found to be sufficiently high to be used in the following reactions with the phosphate nucleophiles.

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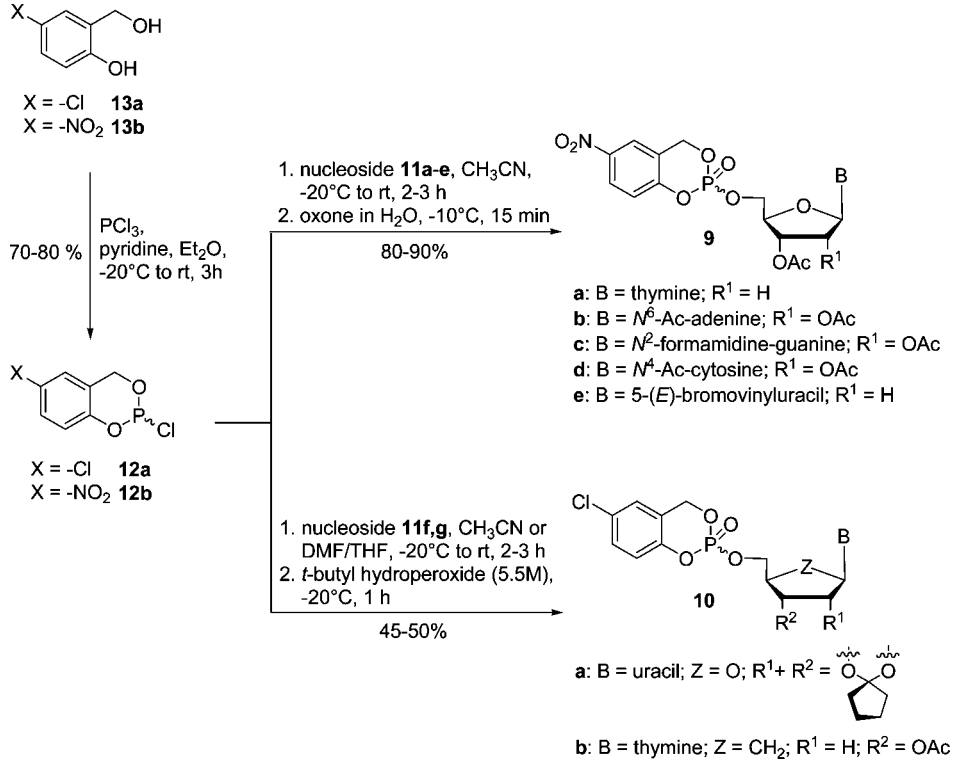
SCHEME 2. Synthesis of *cyclo*Sal-nucleotides 9 and 10



The commercially available sodium phosphates (Na_2HPO_4, $Na_2H_2P_2O_7$) as well as Na_2UMP and Na_2H_2ATP were converted into their corresponding $(n\text{-Bu})_4N^+$ salts **5**−**8** by ion-exchange chromatography to increase their nucleophilicity and the solubility in organic solvents.[25] To remove moisture from the hygroscopic solids they were dried in vacuo and further dried over molecular sieves in DMF.

Best results for the reaction of *cyclo*Sal-nucleotides **9** and **10** and phosphate or pyrophosphate salts **5** and **6** were obtained using DMF as solvent. *Cyclo*Sal-triesters **9** and **10** were completely converted after 3−5 h at room temperature (Scheme 3).

Prior to the purification the *N*,*O*-protecting groups were cleaved. The acetal protecting group was removed with catalytic amounts of HCl while the acetyl and formamidine (in **17**) groups were cleaved by treating the crude product with a mixture of NEt_3/MeOH/H_2O 1:7:3. Although salts of **5** and **6** acted very efficiently in the reactions, the resulting salts of the ND(T)Ps show very difficult chromatographic properties. To overcome this hurdle, the $(n\text{-Bu})_4N^+$ ions were replaced by NH_4^+ ions before chromatography by ion-exchange with cation-exchange resin. The ND(T)Ps were finally purified by chromatography on RP-18 silica gel in a glass column using pure water as eluting solvent. After purification of the nucleoside di- and triphosphates **14**−**23**, yields between 40% and 83% were obtained (Table 1). From the obtained yields it can be concluded that the 5-nitro-substituted *cyclo*Sal-phosphate triesters **9** should be used preferentially. However, here also the 5-Cl-substituted derivatives **10** led to the target compounds in good chemical yields. The variations in the reported yields are most probably due to differences in the chromatographic properties of compounds **14**−**23** because the reaction itself proceeded in all cases equally well.

TABLE 1. Yields of the Synthesized NTPs and NDPs 14−23

product	yield (%)	product	yield (%)
14	83	**19**	56
15	80	**20**	52
16	65	**21**	55
17	40	**22**	50
18	43	**23**	40

The new method was applied for the synthesis of two non-natural nucleoside di- and triphosphates. Since *carba*-dT showed very promising anti-HIV-1 and HIV-2 properties,[26] the corresponding triphosphate **23** was needed for mechanistic studies with viral DNA polymerases. These studies revealed a unique mechanism of action ("kinetic chain termination").[27] In addition, the di- and triphosphate of the highly anti-HSV-1 and varicella zoster virus (VZV) active nucleoside analogue BVDU[28] **19** and **20** were prepared as analytical reference compounds for metabolism studies.

Finally, the *cyclo*Sal approach was used for the successful and efficient synthesis of asymmetric dinucleoside polyphosphates. Up_2T **24** and Ap_4T **25** were synthesized by reaction of 5-nitro-*cyclo*Sal-thymidine triester **9a** with freshly prepared and rigorously dried $(n\text{-Bu})_4$N-UMP **7** and $[(n\text{-Bu})_4N]_3$-ATP **8** in DMF at room temperature within 3−5 h (Scheme 4).

In contrast to the preparation of NDPs or NTPs, for a rapid and efficient formation of the dinucleoside di- or tetraphosphates **24**,**25** it is absolutely necessary to use 5-nitro-substituted *cyclo*Sal-nucleotides. After workup, deprotection, and ion-exchange (n-NBu_4^+ to NH_4^+) products **24** and **25** were purified again on RP-18 silica gel. The isolated yields were found to be between 52% and 60%.

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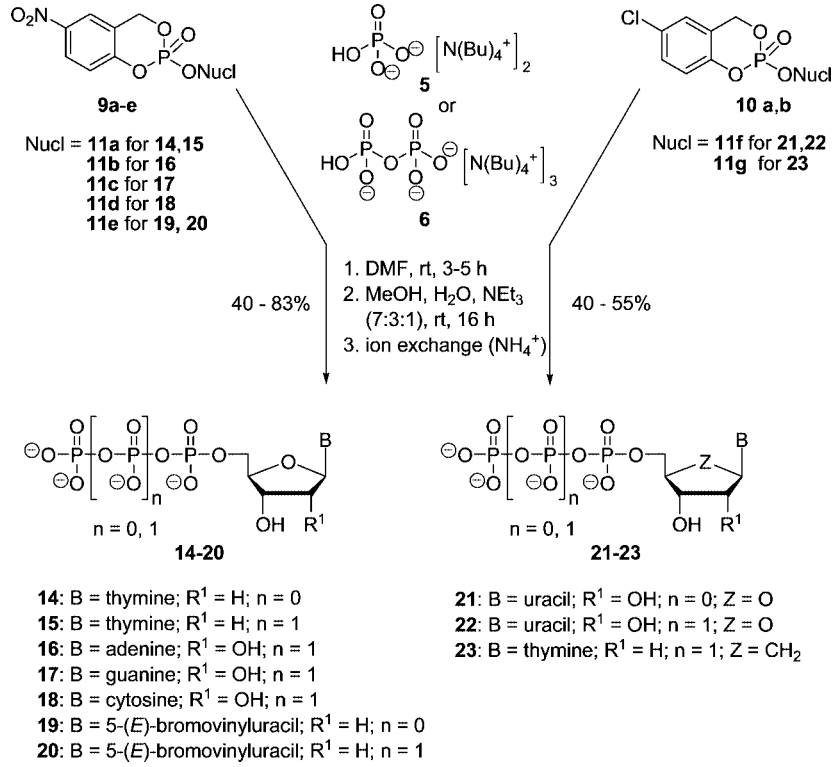
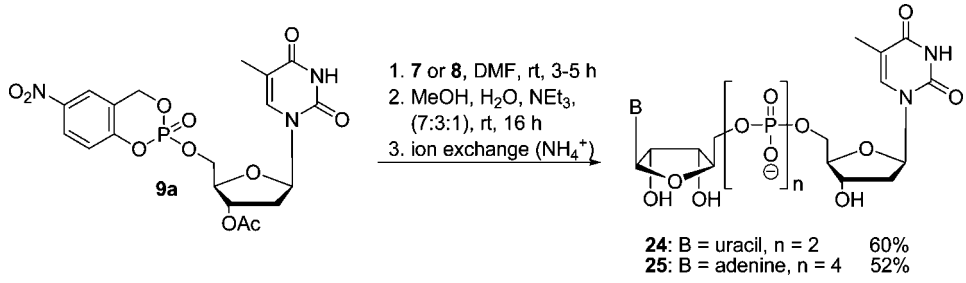
SCHEME 3. Synthesis of NTP and NDP 14−23 Starting from *cyclo*Sal-phosphate Triesters



14: B = thymine; R^1 = H; n = 0
15: B = thymine; R^1 = H; n = 1
16: B = adenine; R^1 = OH; n = 1
17: B = guanine; R^1 = OH; n = 1
18: B = cytosine; R^1 = OH; n = 1
19: B = 5-(*E*)-bromovinyluracil; R^1 = H; n = 0
20: B = 5-(*E*)-bromovinyluracil; R^1 = H; n = 1

21: B = uracil; R^1 = OH; n = 0; Z = O
22: B = uracil; R^1 = OH; n = 1; Z = O
23: B = thymine; R^1 = H; n = 1; Z = CH_2

SCHEME 4. Synthesis of Dinucleoside Polyphosphates 24 and 25



24: B = uracil, n = 2 60%
25: B = adenine, n = 4 52%

Conclusion

In summary, we have developed an effective, fast, and reliable method for the preparation of di- and triphosphates of natural nucleosides that is at least complementary to other reported procedures. In addition, the same approach was applied successfully to the synthesis of dinucleoside polyphosphates. Our protocol involves the reaction of 5-acceptor-substituted *cyclo*Sal-nucleotides (preferentially with the 5-nitro group) as active esters with phosphate, pyrophosphate, or nucleotides. Thus, the reported procedures can be used in general as an approach for the synthesis of these interesting classes of natural compounds. Moreover, this chemical method proved to be suitable for the synthesis of (poly)phosphates of nucleoside analogues which offers an efficient access of these derivatives for, e.g., biochemical studies.

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Experimental Section

Synthesis, Isolation, and Characterization of Compounds 9a−e, 10a,b, 23, 24, and 25. General Procedure for the Synthesis of 5-Nitro-Substituted *cyclo*Sal-nucleotides. (N),O-Ac-protected nucleosides (1.0 equiv) were dissolved in acetonitrile, and the solution was cooled to −20 °C. Then diisopropylethylamine (DIPEA, 2.0 equiv) and 5-nitrocyclosaligenylchlorophosphite **12b** (2.0 equiv.), dissolved in anhydrous acetonitrile were added. The reaction mixture was warmed to room temperature, and stirring was continued for 3−4 h. Subsequently, Oxone (4.0 equiv) dissolved in water was added at −10 °C. Cooling was removed, and stirring was continued for 15 min. The mixture was then directly extracted with ethyl acetate and cold water (2×), the organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure. The resulting solid was resolved in dichloromethane. After filtration from the precipitate, the solvent was again removed in vacuo. Lyophilization yielded the products as colorless foams. The triesters obtained were of high purity and could be used without further purification.

Preparation of 5-Nitro-*cyclo*Sal-3′-*O*-acetylthymidine Monophosphate (9a). Compounds **11a** (0.49 g, 1.7 mmol), **12b** (0.80 g, 3.4 mmol), DIPEA (0.60 mL, 3.4 mmol), Oxone (4.2 g, 6.8 mmol), and acetonitrile (50 mL) were used. Yield: 91% (700 mg, 1.55 mmol) of a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ =

11.35 (bs, 2H, 2 × N*H*), 8.30 (d, 2H, $^4J_{HH}$ = 1.9 Hz, 2 × H6), 8.26 − 8.22 (m, 2H, 2 × $H4_{ar}$), 7.49 (d, 1H, $^4J_{HH}$ = 1.0 Hz, $H6_{ar}$), 7.47 (d, 1H, $^4J_{HH}$ = 1.0 Hz, $H6_{ar}$), 7.38 (d, 1H, $^3J_{HH}$ = 8.8 Hz, $H3_{ar}$), 7.38 (d, 1H, $^3J_{HH}$ = 8.8 Hz, $H3_{ar}$), 6.15 (dd, 1H, $^3J_{HH}$ = 8.2 Hz, $^3J_{HH}$ = 6.0 Hz, H1′), 6.12 (dd, 1H, $^3J_{HH}$ = 8.2 Hz, $^3J_{HH}$ = 6.0 Hz, H1′), 5.70 − 5.53 (m, 4H, 2 × CH_2-benzyl), 5.17 − 5.15 (m, 2H, 2 × H3′), 4.46 − 4.36 (m, 4H, 2 × H5′), 4.17−4.15 (m, 2H, 2 × H4′), 2.42−2.33 (m, 2H, 2 × H2'a), 2.25 (ddd, 2H, $^2J_{HH}$ = 7.9 Hz, $^3J_{HH}$ = 5.35 Hz, $^3J_{HH}$ = 6.0 Hz, 2 × H2′b), 2.05 (s, 3H, CH_3-acetyl), 2.04 (s, 3H, CH_3-acetyl), 1.76 (s, 3H, H7), 1.73 (s, 3H, H7) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 168.9 (2 × C_q-acetyl), 163.6 (2 × C4), 156.0 (2 × $C5_{ar}$), 150.3 (2 × C2), 143.5 (d, $^2J_{CP}$ = 6.1 Hz, 2 × $C2_{ar}$), 135.9 (C6), 135.8 (C6), 125.5 ($C4_{ar}$), 125.4 ($C4_{ar}$), 122.6 (2 × $C6_{ar}$), 119.7 (d, $^3J_{CP}$ = 9.1 Hz, 2 × $C1_{ar}$), 119.6 (d, $^3J_{CP}$ = 8.8 Hz, 2 × $C3_{ar}$), 109.9 (2 × C5), 84.2 (2 × C1′), 81.4 (d, $^3J_{CP}$ = 7.2 Hz, C4′), 81.3 (d, $^3J_{CP}$ = 7.2 Hz, C4′), 73.2 (2 × C3′), 68.0 (d, $^2J_{CP}$ = 7.2 Hz, 2 × C-benzyl), 67.9 (d, $^2J_{CP}$ = 6.1 Hz, 2 × C5′), 35.5 (C2′), 35.4 (C2′), 20.7 (2 × CH_3-acetyl), 12.0 (2 × C7) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −10.63 (s), −10.81 (s) (2 diastereomers in a ratio of 1:1) ppm. MS-FAB (*m*/*z*): calcd 498.1 (M + H$^+$), found 498.1.

Preparation of 5-Nitro-*cyclo*Sal-N^6-acetyl-2′,3′-*O*-acetyladenosine Monophosphate (9b). Compound **11b** (0.25 g, 0.64 mmol), **12b** (0.30 g, 1.3 mmol), DIPEA (0.22 mL, 1.3 mmol), Oxone (1.56 g, 2.54 mmol), and acetonitrile (20 mL) were used. Yield: 80% (0.31 g, 0.51 mmol) of a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.74 (s, 1H, NH), 10.71 (s, 1H, NH), 8.61 (s, 1H, H8), 8.60 (s, 1H, H8), 8.58 (s, 1H, H2), 8.56 (s, 1H, H2), 8.18−8.11 (m, 4H, 2 × $H4_{ar}$, 2 × $H6_{ar}$), 7.23 (d, 1H, $^3J_{HH}$ = 8.9 Hz, $H3_{ar}$), 7.17 (d, 1H, $^3J_{HH}$ = 8.9 Hz, $H3_{ar}$), 6.30 − 6.27 (m, 2H, 2 × H1′), 5.99 (dd, 2H, $^3J_{HH}$ = 9.7 Hz, $^3J_{HH}$ = 5.1 Hz, 2 × H2′), 5.69 − 5.38 (m, 6H, 2 × H3′, 2 × CH_2-benzyl), 4.55 − 4.43 (m, 6H, 2 × H4′, 2 × H5′), 2.26 (s, 3H, NHAc), 2.25 (s, 3H, NHAc), 2.11 (s, 3H, CH_3-acetyl), 2.09 (s, 3H, CH_3-acetyl), 2.03 (s, 3H, CH_3-acetyl), 2.02 (s, 3H, CH_3-acetyl) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 169.3 (2 × C_q-acetyl), 169.2 (2 × C_q-acetyl), 168.8 (2 × C_q-acetyl), 153.8 (d, $^2J_{CP}$ = 7.0 Hz, 2 × $C2_{ar}$), 152.6 (2 × C2), 151.8 (2 × C6), 151.2 (2 × C4), 143.4 (2 × $C5_{ar}$), 125.3 ($C6_{ar}$), 125.2 ($C6_{ar}$), 123.5 (d, $^3J_{CP}$ = 11.6 Hz, 2 × $C1_{ar}$), 122.3 (2 × $C4_{ar}$), 121.9 (2 × C5), 119.3 (d, $^3J_{CP}$ = 9.3 Hz, 2 × $C3_{ar}$), 85.9 (2 × H1′), 79.7 (d, $^3J_{CP}$ = 6.9 Hz, 2 × C4′), 71.9 (C2′), 71.8 (C2′), 69.2 (C3′), 69.1 (C3′), 67.9 (2 × C-benzyl), 66.8 (d, $^2J_{CP}$ = 6.2 Hz, 2 × C5′), 24.3 (NH-CH_3-acetyl), 24.2 (NH-CH_3-acetyl), 20.3 (2 × CH_3-acetyl), 20.1 (2 × CH_3-acetyl) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −10.97 (s), −11.33 (s) (2 diastereomers in a ratio of 1:1) ppm. MS-FAB (*m*/*z*): calcd 607.1 (M + H$^+$), found 607.1.

Preparation of 5-Nitro-*cyclo*Sal-N^2-formamidine-2′,3′-*O*-acetylguanosine Monophosphate (9c). Compound **11c** (0.20 g, 0.47 mmol), **12b** (0.22 g, 0.94 mmol), DIPEA (0.16 mL, 0.94 mmol), Oxone (1.15 g, 1.88 mmol), and acetonitrile (20 mL) were used. Yield: 83% (0.25 g, 0.39 mmol) of a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.39 (bs, 1H, NH), 11.25 (bs, 1H, NH), 8.60 (s, 1H, CH-formamidine), 8.59 (s, 1H, H10), 8.21−8.15 (m, 4H, 2 × $H4_{ar}$, 2 × $H6_{ar}$), 7.91 (s, 1H, H8), 7.90 (s, 1H, H8), 7.21 (d, 1H, $^3J_{HH}$ = 4.9 Hz, $H3_{ar}$), 7.20 (d, 1H, $^3J_{HH}$ = 4.9 Hz, $H3_{ar}$), 6.09 (d, 2H, $^3J_{HH}$ = 5.1 Hz, 2 × H1′), 5.98 (dd, 2H, $^3J_{HH}$ = 4.77 Hz, 2 × H2′), 5.92−5.75 (m, 2H, H3′), 5.59−5.32 (m, 4H, 2 × CH_2-benzyl), 4.52−4.31 (m, 6H, 2 × H4′, 2 × H5′), 3.13 (s, 6H, 2 × CH_3-formamidine), 3.03 (s, 6H, 2 × CH_3-formamidine), 2.08 (s, 6H, 2 × CH_3-acetyl), 2.07 (s, 6H, 2 × CH_3-acetyl) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 169.4 (2 × C_q-acetyl), 157.9 (2 × CH-formamidine), 157.3 (2 × C6, 2 × C2), 149.2 (2 × C4), 149.0 (2 × $C2_{ar}$), 143.4 (2 × $C5_{ar}$), 138.2 (C8), 138.0 (C8), 125.5 ($C6_{ar}$), 125.4 ($C6_{ar}$), 122.3 ($C4_{ar}$), 122.1 ($C4_{ar}$), 121.9 (2 × $C1_{ar}$), 120.2 (2 × C5), 119.3 ($C3_{ar}$), 119.2 ($C3_{ar}$), 86.6 (C1′), 86.4 (C1′), 78.5 (d, $^3J_{CP}$ = 7.3 Hz, 2 × C4′), 72.5 (C3′), 72.3 (C3′), 67.7 (C2′), 67.5 (C2′), 66.4 (2 × C-benzyl), 65.8 (2 × C5′), 40.5 (CH_3-formamidine), 34.7 (CH_3-formamidine), 20.3 (2 × CH_3-acetyl) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −10.46 (s), −10.63 (s) (2 diastereomers in a ratio of 1:1) ppm. MS-FAB (*m*/*z*): calcd 636.1 (M + H$^+$), found 636.1.

Preparation of 5-Nitro-*cyclo*Sal-N^4-acetyl-2′,3′-*O*-acetylcytosine Monophosphate (9d). Compound **11d** (0.474 g, 1.28 mmol), **12b** (600 mg, 2.56 mmol), DIPEA (0.450 mL, 2.56 mmol), Oxone (3.21 g, 5.12 mmol) and acetonitrile (30 mL) were used. Yield: 83% (620 mg, 1.06 mmol) of a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.19 (bs, 2H, 2 × N*H*), 8.01 (d, 2H, $^3J_{HH}$ = 7.3 Hz, 2 × H6), 7.40 − 7.33 (m, 4H, 2 × $H3_{ar,}$ 2 × H5), 7.17−7.13 (m, 4H, 2 × $H4_{ar}$, 2 × $H6_{ar}$), 5.86 (d, 1H, $^3J_{HH}$ = 3.7 Hz, H1′), 5.81 (d, 1H, $^3J_{HH}$ = 3.7 Hz, H1′), 5.50−5.45 (m, 4H, 2 × CH_2-benzyl), 5.41 (dd, 2H, $^3J_{HH}$ = 6.3 Hz, $^3J_{HH}$ = 6.3 Hz, 2 × H2′), 5.32 (dd, 2H, $^3J_{HH}$ = 6.3 Hz, $^3J_{HH}$ = 6.3 Hz, 2 × H3′), 4.50 − 4.29 (m, 6H, 2 × H4′, 2 × H5′), 2.12 (s, 6H, 2 × NH-CH_3-acetyl), 2.11 (s, 3H, CH_3-acetyl), 2.06 (s, 3H, CH_3-acetyl), 2.04 (s, 3H, CH_3-acetyl), 2.01 (s, 3H, CH_3-acetyl) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 171.0 (C_q-acetyl), 169.3 (4 × C_q-acetyl), 169.2 (C_q-acetyl), 163.0 (2 × C2), 154.1 (2 × C4), 146.7 (C6), 146.6 (C6), 129.4 (2 × $C4_{ar}$), 128.3 (d, $^2J_{CP}$ = 8.9 Hz, 2 × $C2_{ar}$), 128.2 (2 × $C5_{ar}$), 126.1 (d, $^3J_{CP}$ = 6.2 Hz, 2 × $C1_{ar}$), 125.6 (2 × $C6_{ar}$), 120.0 (d, $^3J_{CP}$ = 8.4 Hz, 2 × $C3_{ar}$), 91.3 (C5), 91.0 (C5), 79.3 (2 × C1′), 84.2 (2 × C1′), 72.5 (d, $^3J_{CP}$ = 7.6 Hz, 2 × C4′), 69.2 (2 × C2′), 68.9 (2 × C3′), 68.1 (d, $^2J_{CP}$ = 6.6 Hz, 2 × C-benzyl), 66.8 (d, $^2J_{CP}$ = 5.1 Hz, C5′), 66.5 (d, $^2J_{CP}$ = 5.1 Hz, C5′), 29.7 (2 × NH-CH_3-acetyl), 20.3 (4 × CH_3-acetyl) ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = −10.02 (s), −10.29 (s) (2 diastereomers in a ratio of 1:1) ppm. MS-FAB (*m*/*z*): calcd 583.1 (M + H$^+$), found 583.1.

Preparation of 5-Nitro-*cyclo*Sal-3′-*O*-acetylBVDU Monophosphate (9e). Compound **11e** (0.20 g, 0.53 mmol), **12b** (249 mg, 1.07 mmol), DIPEA (0.187 mL, 1.07 mmol), Oxone (1.31 g, 2.14 mmol), and acetonitrile (30 mL) were used. Yield: 87% (0.28 g, 0.46 mmol) of a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.62 (bs, 2H, 2 × N*H*), 7.80 (s, 1H, H6), 7.78 (s, 1H, H6), 7.35−7.30 (m, 4H, 2 × $H4_{ar}$, 2 × $H6_{ar}$), 7.28 (d, 1H, $^3J_{HH}$ = 13.6 Hz, H8), 7.27 (d, 1H, $^3J_{HH}$ = 13.6 Hz, H8), 7.14 (d, 1H, $^3J_{HH}$ = 8.5 Hz, $H3_{ar}$), 7.12 (d, 1H, $^3J_{HH}$ = 8.5 Hz, $H3_{ar}$), 6.81 (d, 1H, $^3J_{HH}$ = 13.6 Hz, H7), 6.80 (d, 1H, $^3J_{HH}$ = 13.6 Hz, H7), 6.19 (dd, 1H, $^3J_{HH}$ = 7.6 Hz, $^3J_{HH}$ = 6.1 Hz, H1′), 6.18 (dd, 1H, $^3J_{HH}$ = 7.6 Hz, $^3J_{HH}$ = 6.1 Hz, H1′), 5.51−5.39 (m, 4H, 2 × CH_2-benzyl), 5.19−5.14 (m, 2H, 2 × H3′), 4.47−4.31 (m, 4H, 2 × H5′), 4.19−4.16 (m, 2H, 2 × H4′), 2.43−2.24 (m, 4H, 2 × H2′), 2.05 (s, 3H, CH_3-acetyl), 2.04 (s, 3H, CH_3-acetyl) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 169.9 (2 × C_q-acetyl), 161.6 (2 × C4), 149.2 (2 × C2), 145.7 (2 × $C5_{ar}$), 139.3 (C6), 139.2 (C6), 129.6 (2 × C7), 128.3 (2 × $C2_{ar}$), 126.0 (2 × $C4_{ar}$, 2 × $C6_{ar}$), 122.9 (2 × $C1_{ar}$), 120.1 (d, $^3J_{CP}$ = 11.5 Hz, 2 × $C3_{ar}$), 110.3 (C5), 110.2 (C5), 107.2 (C8), 84.9 (C1′), 84.6 (C1′), 81.8 (d, $^3J_{CP}$ = 6.1 Hz, C4′), 73.1 (C3′), 73.0 (C3′), 68.1 (d, $^2J_{CP}$ = 6.9 Hz, 2 × C-benzyl), 67.8 (d, $^2J_{CP}$ = 6.0 Hz, 2 × C5′), 36.0 (C2′), 35.8 (C2′), 20.8 (2 × CH_3-acetyl) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −10.14 (s), −10.32 (s) (2 diastereomers in a ratio of 1:1) ppm. MS-FAB (*m*/*z*): calcd 588.0, 590.0 (M + H$^+$), found 588.2, 590.2.

5-Chloro-*cyclo*Sal-2′,3′-*O*-cyclopentyluridine Monophosphate (10a). Compound **11f** (0.30 g, 0.97 mmol) was dissolved in 20 mL of a mixture of anhydrous THF/DMF (v/v 1:1) and cooled to −20 °C. To this solution were added diisopropylethylamine (DIPEA, 0.340 mL, 1.94 mmol) and 5-chloro-*cyclo*saligenylchlorophosphite **12a** (433 mg, 1.94 mmol), dissolved in anhydrous THF. The reaction mixture was warmed to room temperature, and stirring was continued for 2−3 h. Subsequently, *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 1.94 mmol, 0.35 mL) was added at −20 °C. Cooling was removed, and the stirring was continued for 1 h. The solvent was removed under reduced pressure, and the resulting residue was purified by preparative TLC (Chromatotron, (1) ethyl acetate/methanol (9:1), (2) dichloromethane/methanol gradient). Lyophilization yielded the product as a colorless foam. Yield: 50% (0.25 g, 0.49 mmol) of a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.43 (bs, 2H, 2 × N*H*), 7.68 (d, 1H, $^3J_{HH}$ = 8.0 Hz, H6), 7.63 (d, 1H, $^3J_{HH}$ = 8.0 Hz, H6), 7.44−7.38 (m,

4H, 2 × $H4_{ar}$, 2 × $H6_{ar}$), 7.17 (d, 1H, $^3J_{HH}$ = 9.0 Hz, $H3_{ar}$), 7.12 (d, 1H, $^3J_{HH}$ = 9.0 Hz, $H3_{ar}$), 5.77 (d, 1H, $^3J_{HH}$ = 1.9 Hz, H1′), 5.75 (d, 1H, $^3J_{HH}$ = 1.9 Hz, H1′), 5.60 (d, 1H, $^3J_{HH}$ = 8.0 Hz, H5), 5.58 (d, 1H, $^3J_{HH}$ = 8.0 Hz, H5), 5.53 − 5.39 (m, 4H, 2 × CH_2-benzyl), 4.99 (dd, 1H, $^3J_{HH}$ = 6.6 Hz, $^3J_{HH}$ = 6.3 Hz, H2′), 4.96 (dd, 1H, $^3J_{HH}$ = 6.6 Hz, $^3J_{HH}$ = 6.3 Hz, H2′), 4.72 − 4.70 (m, 2H, 2 × H3′), 4.37−4.25 (m, 4H, 2 × H5′), 4.21−4.18 (m, 2H, 2 × H4′), 2.05−2.02 (m, 4H, 2 × H7a, 2 × H10a), 1.85−1.81 (m, 4H, 2 × H7b, 2 × H10b), 1.74−1.67 (m, 8H, 2 × H8, 2 × H9) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 163.2 (2 × C4), 156.3 (2 × $C5_{ar}$), 150.3 (C2), 150.2 (C2), 148.2 (d, $^2J_{CP}$ = 6.3 Hz, 2 × $C2_{ar}$), 143.4 (C6), 143.3 (C6), 129.5 ($C4_{ar}$), 129.4 ($C4_{ar}$), 128.2 (2 × C7), 126.0 (2 × $C6_{ar}$), 122.5 (d, $^3J_{CP}$ = 9.1 Hz, 2 × $C1_{ar}$), 120.2 (d, $^3J_{CP}$ = 5.1 Hz, $C3_{ar}$), 120.1 (d, $^3J_{CP}$ = 5.1 Hz, $C3_{ar}$), 101.8 (C5), 101.7 (C5), 93.3 (C1′), 93.2 (C1′), 84.7 (d, $^3J_{CP}$ = 7.7 Hz, 2 × C4′), 83.4 (2 × C2′), 80.4 (C3′), 80.3 (C3′), 68.1 (d, $^2J_{CP}$ = 7.2 Hz, 2 × C-benzyl), 67.8 (d, $^2J_{CP}$ = 6.8 Hz, 2 × C5′), 35.8 (C7, C10), 35.7 (C7, C10), 22.9 (2 × C8), 22. Si × (2 × C9) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −10.43 (s), −10.63 (s) (2 diastereomers in a ratio of 1:1) ppm. HRMS-ESI (*m*/*z*): calcd 535.0649 [M + Na], found 535.0652.

5-Chloro-*cyclo*Sal-3′-*O*-acetyl-2′-deoxy-6′-*carba*-thymidine Monophosphate (10b). This compound was synthesized and purified in the same way as described for *cyclo*Sal-nucleotide **10a**: **11g** (0.22 g, 0.76 mmol), **12a** (0.26 g, 1.2 mmol), DIPEA (0.26 mL, 1.5 mmol), *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 0.27 mL, 1.5 mmol), and THF/DMF (v/v 1:1, 15 mL) were used. Yield: 40% (0.14 g, 0.29 mmol) of a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.21 (bs, 2H, 2 × N*H*), 7.54 (d, 2H, $^4J_{HH}$ = 0.8 Hz, 2 × H6), 7.43−7.40 (m, 4H, 2 × $H4_{ar}$, 2 × $H6_{ar}$), 7.19 (d, 1H, $^3J_{HH}$ = 7.6 Hz, $H3_{ar}$), 7.17 (d, 1H, $^3J_{HH}$ = 7.6 Hz, $H3_{ar}$), 5.55−5.39 (m, 4H, 2 × CH_2-benzyl), 4.99−4.95 (m, 2H, 2 × H3′), 4.87−4.82 (m, 2H, 2 × H1′), 4.30−4.16 (m, 4H, 2 × H5′), 2.39−2.32 (m, 2H, 2 × H4′), 2.18−2.02 (m, 4H, 2 × H2′), 1.97 (s, 3H, CH_3-acetyl), 1.94 (s, 3H, CH_3-acetyl), 1.92−1.85 (m, 2H, 2 × H6′a) 1.77 (d, 3H, $^4J_{HH}$ = 0.8 Hz, H7), 1.76 (d, 3H, $^4J_{HH}$ = 0.8 Hz, H7), 1.56−1.45 (m, 2H, 2 × H6′b) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 172.6 (2 × C_q-acetyl), 163.7 (2 × C4), 150.8 (2 × C2), 148.3 (2 × $C2_{ar}$), 137.8 (C6), 137.7 (C6), 129.5 (2 × $C4_{ar}$), 128.2 (2 × $C5_{ar}$) 125.9 (2 × $C6_{ar}$), 121.4 (2 × $C1_{ar}$), 120.1 (d, $^3J_{CP}$ = 8.8 Hz, 2 × $C3_{ar}$), 109.2 (2 × C5), 74.1 (C3′), 74.0 (C3′) 69.0 (d, $^2J_{CP}$ = 6.8 Hz, 2 × C-benzyl), 68.0 (d, $^2J_{CP}$ = 7.6 Hz, C5′), 53.2 (C1′), 53.1 (C1′), 43.9 (C4′), 43.8 (C4′), 35.7 (C2′), 35.6 (C2′), 31.4 (C6′), 31.3 (C6′), 20.8 (CH_3-acetyl), 20.7 (CH_3-acetyl) 12.0 (2 × C7) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −10.46 (s), −10.52 (s) (2 diastereomers in a ratio of 1:1) ppm. HRMS-ESI (*m*/*z*): calcd 507.0700 (M + Na), found 507.0704.

General Procedure for the Synthesis of Nucleoside Di- and Triphosphates. Freshly prepared bis(tetra-*n*-butylammonium) hydrogen phosphate or tris(tetra-*n*-butylammonium) hydrogen pyrophosphate (2.0 equiv) were dried in vacuo for 2 h and further dried for 2 h in anhydrous DMF over activated molecular sieves (4 Å). This solution was added dropwise to a solution of the *cyclo*Sal-nucleotide (1.0 equiv) in DMF, with stirring at room temperature. The resulting mixture was stirred at the same temperature for 3−5 h; after this time, the solvent was removed under reduced pressure and the residue was extracted with ethyl acetate and water two times. The combined aqueous layer was dried by lyophilization. Cleavage of the acetal group: The remaining layer was resolved in water, and 5 drops of concentrated HCl were added with stirring, the mixture was refluxed for 1 min and dried again by lyophilization. Cleavage of the acetyl groups: The remaining layer was resolved in a mixture of methanol (120 equiv), water (51 equiv), and triethylamine (17 equiv) and stirred at room temperature overnight. Before lyophilization, methanol was removed in vacuo, and the mixture was diluted with water. The residue was dissolved in water and applied to a column filled with ion-exchange resin in ammonia form. After lyophilization, the crude product was purified on RP-18 silica gel in a glass column using water as eluent.

Preparation of *carba*-dTTP (23). Tris(tetra-*n*-butylammonium) hydrogen pyrophosphate (433 mg, 0.48 mmol), **10b** (0.12 g, 0.24 mmol), and 10 mL of DMF were stirred at room temperature for 3 h. Yield: 40% (71 mg, 0.12 mmol) of a colorless solid. ^{1}H NMR (400 MHz, D_2O): δ = 7.54 (d, 1H, $^4J_{HH}$ = 1.0 Hz, H6), 5.03−4.98 (m, 1H, H1′), 4.35−4.31 (m, 1H, H3′), 4.08−3.99 (m, 2H, H5′), 3.16 (q, 6H, $^3J_{HH}$ = 7.3 Hz, CH_2-TEA), 2.26−2.19 (m, 2H, H6′a, H4′), 2.15−2.04 (m, 1H, H2′a), 2.01 (ddd, 1H, $^2J_{HH}$ = 10.8 Hz, $^3J_{HH}$ = 8.6 Hz, $^3J_{HH}$ = 4.3 Hz, H2′b), 1.85 (d, $^4J_{HH}$ = 1.0 Hz, H7), 1.62 (dd, 1H, $^2J_{HH}$ = 11.8 Hz, $^3J_{HH}$ = 6.8 Hz, H6′b), 1.24 (t, 9H, $^3J_{HH}$ = 7.3 Hz, CH_3-TEA) ppm. ^{13}C NMR (101 MHz, D_2O): δ = 167.0 (C4), 152.8 (C2), 140.2 (C6), 111.5 (C5), 72.5 (C3′), 67.3 (d, $^2J_{CP}$ = 5.6 Hz, C5′), 54.5 (C1′), 47.1 (CH_2-TEA), 47.0 (d, $^3J_{CP}$ = 8.7 Hz, C4′), 38.4 (C2′), 32.2 (C6′), 11.8 (C7), 8.7 (CH_3-TEA) ppm. ^{31}P NMR (162 MHz, D_2O): δ = −9.22 (d, 1P, $^2J_{PP}$ = 19.8 Hz, P_γ), −10.74 (d, 1P, $^2J_{PP}$ = 19.8 Hz, P_α), −22.43 (dd, 1P, $^2J_{PP}$ = 19.8 Hz, $^2J_{PP}$ = 19.8 Hz, P_β) ppm. HRMS-ESI (*m*/*z*): calcd 479.0022 [M − H]$^-$, found 479.0027.

Preparation of Up_2T (24). Freshly prepared (tetra-*n*-butylammonium) hydrogen uridine monophosphate (112 mg, 0.2 mmol, 2.0 equiv) was dried in vacuo for 2 h and further dried for 2 h in 5 mL of anhydrous DMF over activated molecular sieves 4 Å. This solution was added dropwise to a solution of the *cyclo*Sal-nucleotide **9a** (50 mg, 0.1 mmol, 1.0 equiv) in 5 mL of DMF. The reaction mixture was stirred at room temperature for 5 h. The solvent was removed under reduced pressure, and the residue was extracted with ethyl acetate and water twice. The combined aqueous layer are dried by lyophilization. The acetyl group was cleaved as described above. After ion-exchange chromatography [(n-Bu)$_4$N$^+$ to NH_4^+], the crude product was purified on RP-18 silica gel in a glass column using water as eluent. Yield 60% (44 mg, 0.06 mmol) of a colorless solid. ^{1}H NMR (400 MHz, D_2O): δ = 7.83 (d, 1H, $^2J_{HH}$ = 8.0 Hz, H6-U), 7.65 (d, 1H, $^4J_{HH}$ = 1.2 Hz, H6-T) 6.27−6.23 (m, 1H, H1′-T) 5.85−5.83 (m, 2H, H1′-U, H5-U), 5.63−4.60 (m, 1H, H3′-T), 4.26−4.24 (m, 3H, H4′-T, H2′-U, H3′-U), 4.16−4.08 (m, 5H, H4′-U, H5′-U, H5′-T), 2.35−2.32 (m, 2H, H2′-T), 2.15−2.04 (m, 1H, H2′a), 1.82 (s, 3H, H7-T) ppm. ^{13}C NMR (101 MHz, D_2O): δ = 166.4 (C4-T), 166.0 (C4-U), 151.7 (2 × C2 U+T), 141.6 (C6-U), 137.3 (C6-T), 111.9 (C5-T), 102.7 (C5-U), 88.4 (C1′-U), 84.8 (C1′-T), 83.1 (d, $^3J_{CP}$ = 8.1 Hz, 2 × C4′U+T) 73.6 (C3′-T), 73.1 (C3′-U), 69.6 (C2′-U), 65.8 (d, $^2J_{CP}$ = 5.2 Hz, C5′-T), 64.8 (d, $^2J_{CP}$ = 5.2 Hz, C5′-U), 36.4 (C2′-T), 11.7 (C7-T) ppm. ^{31}P NMR (162 MHz, D_2O): δ = −11.43 (d, 1P, $^2J_{PP}$ = 21.0 Hz,), −11.60 (d, 1P, $^2J_{PP}$ = 21.0 Hz,) ppm. HRMS-ESI (*m*/*z*): calcd 628.0819 [M], found 628.0812.

Preparation of Ap_4T (25). Freshly prepared tris(tetra-*n*-butylammonium) hydrogen adenosine triphosphate (300 mg, 0.24 mmol, 2.0 equiv) was dried in vacuo for 2 h and further dried for 2 h in 5 mL of anhydrous DMF over activated molecular sieves 4 Å. This solution was added dropwise to a solution of *cyclo*Sal-nucleotide **9a** (60 mg, 0.12 mmol, 1.0 equiv) in 5 mL of DMF. The reaction mixture was stirred at room temperature for 5 h. The solvent was removed under reduced pressure, and the residue was extracted with ethyl acetate and water twice. The combined aqueous layers were dried by lyophilization. The acetyl group was cleaved as described above. After ion-exchange chromatography [(n-Bu)$_4$N$^+$ to NH_4^+], the crude product was purified on RP-18 silica gel in a glass column using water as eluent. Yield: 52% (55 mg, 0.062 mmol) of a colorless solid. ^{1}H NMR (400 MHz, D_2O): δ = 8.56 (s, 1H, H8-A), 8.29 (s, 1H, H2-A), 7.67 (s, 1H, H6-T), 6.22−6.19 (m, 1H, H1′-T), 6.06−6.05 (d, 1H, $^3J_{HH}$ = 6.0 Hz, H1′-A), 5.35−5.34 (m, 1H, H3′-T), 4.63−4.61 (m, 1H, H2′-A), 4.55−4.53 (m, 1H, H3′-A), 4.36−4.35 (m, 1H, H4′-A), 4.28−4.27 (m, 1H, H4′-T), 4.26−4.19 (m, 4H, H5′-A, H5′-T), 2.39−2.27 (m, 2H, H2′-T), 1.82 (s, 3H, H7-T) ppm. ^{13}C NMR (101 MHz, D_2O): δ = 166.7 (C4-T), 152.8 (C2-A), 151.9 (C6-A), 151.7 (C2 T), 148.9 (C4-A), 140.4 (C8-A), 137.5 (C6-T), 120.3 (C5-A), 112.2 (C5-T), 87.6 (C1′-A), 85.1 (C1′-T), 84.6 (d, $^3J_{CP}$ = 9.2 Hz, C4′-A), 83.4 (d, $^3J_{CP}$ = 8.4 Hz, C4′-T), 74.1 (C3′-T), 70.8 (C2′-A), 70.0 (C3′-A), 66.2 (d, $^2J_{CP}$ = 5.4 Hz, C5′-A), 65.5 (d, $^2J_{CP}$ = 4.7 Hz, C5′-T), 36.9 (C2′-T),

12.0 (C7-T) ppm. ^{31}P NMR (162 MHz, D_2O): $\delta = -22.59$ (2P), -11.35 (1P), -11.15 (1P) ppm. HRMS-ESI (*m*/*z*): calcd 810.0345 $[M - H]^-$, found 810.0343.

Acknowledgment. This work was supported by the University of Hamburg; S.W. is grateful to the University of Hamburg for a scholarship.

Supporting Information Available: ^{1}H, ^{13}C, and ^{31}P NMR spectra for compounds **10a** and **14**−**23**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO802348H