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Synthesis of Pyranonucleoside-6′-triphosphates through the *cyclo*Sal-Method

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The high yielding synthesis of pyranonucleoside-6′-triphosphates by using the *cyclo*Sal-method is described. Synthesis of the activated *cyclo*Sal-pyranonucleoside-6′-phosphate triesters was achieved by applying a synthetic route that had been developed for the synthesis of *cyclo*Sal-(glycopyranosyl-6)-phosphates by us. The route involved regioselective 6′-*tert*-butyldimethylsilyl protection and exchange of the silyl protecting group by the fluorenylmethyloxycarbonyl (Fmoc) group. The 6′-Fmoc-protected derivatives were selectively converted into the *cyclo*Sal-triester. These were then very efficiently converted into triphosphates by a "titration-like" reaction with pyrophosphate. Simple purification by first ion exchange followed by reversed phase (RP) column chromatography afforded the triphosphates in very good yields.

Introduction

Nucleoside analogues can exhibit significant antiviral, antibacterial and cytostatic activity. In these analogues modifications can be present in the nucleobase as well as in the glycon moiety. Anti-HIV-active compounds always lack the 3′-OH group of the ribose moiety so that the growing DNA strand – after incorporation of the drug by reverse transcriptase – cannot be further elongated. Other known antiviral active nucleoside analogues contain carbocyclic rings, acyclic skeletons or modified riboses. There have also been reports on the antiviral and cytostatic activity of pyranonucleosides – nucleoside analogues that contain a pyranose moiety instead of a ribose glycon moiety.[1,2] For example, it has been reported that the glucopyranonucleoside 5,6-dibenzyl-1-(β-D-glucopyranosyl)-pyrimidin-2,4(1*H*,3*H*)-dione showed activity against the hepatitis B virus[1] and C5-alkynyl-modified uracil glucopyranonucleosides effectively inhibit tumor cell proliferation of three different tumor cell lines that were tested.[2] Because in vivo nucleoside-5′-triphosphates are the substrates of the enzymes involved in replication (e.g. polymerases as HIV's reverse transcriptase), not the nucleoside analogue itself but its triphosphorylated form is the biologically active compound. To study these enzymes and their interaction with potential drugs, triphosphates of those nucleoside analogues are needed. Although there have already been some reports on enzymatic studies of such triphosphates of nucleosides with six-membered sugars, or analogues thereof,[3–5] little has been reported on the efficient synthesis of these compounds so far. Mainly, the Ludwig method is used.[4–6] Here, a huge excess of pyrophosphate is needed that is often very difficult to separate from the formed triphosphate. This might explain the low yields that were obtained by this method. We have earlier reported on the *cyclo*Sal-method for the preparation of phosphorylated bioconjugates.[7–13] With this method the synthesis of nucleoside-5′-triphosphates (both natural and non-natural compounds) was accomplished in yields of up to 80% starting from the *cyclo*Sal-compound (64% starting from the nucleoside).[8] Herein, the efficient synthesis of pyranonucleoside-6′-triphosphates by the *cyclo*Sal-method is reported, which was achieved by a new "titration-like" reaction that uses only a slight excess of pyrophosphate, which greatly facilitated purification so that the triphosphates were isolated in good yields.

Results and Discussion

Recently, we reported the synthesis of *cyclo*Sal-(glycopyranosyl-6)-phosphates as activated sugar-phosphate precursors for coupling reactions with glycopyranosyl-1-oxides.[14] The synthetic strategy involved a regioselective protection of the 6-position of glycopyranoses with a *tert*-butyldimethylsilyl (TBDMS) group and its selective exchange with a fluorenylmethyloxycarbonyl (Fmoc) group, which was then replaced by the *cyclo*Sal-phosphite in a base-driven reaction. This route was necessary to avoid acetyl group migration to obtain exclusively the 6-phosphorylated compound. Next, we planned to apply this strategy to the synthesis of *cyclo*Sal-(pyranonucleoside-6′)-phosphates for their use as activated phosphate precursors in the synthesis of pyranonucleoside-6′-triphosphates. Therefore, Fmoc-protected precursor **1** was converted into pyranonucleoside

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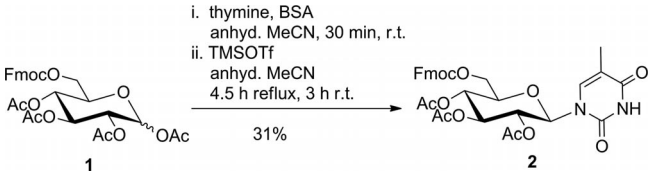
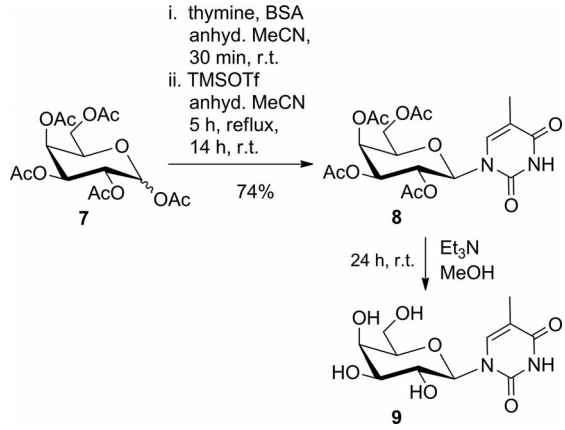
2 by the Vorbrüggen one-pot method:[15,16] The nucleobase was first reacted with *N*,*O*-bis(trimethylsilyl)acetamide in anhydrous acetonitrile in the presence of glycopyranose **1** and then trimethylsilyl trifluoromethanesulfonate (TMSOTf) was added. Compound **2** was obtained in a yield of 31% (Scheme 1).



Scheme 1. First synthesis of 6′-Fmoc-protected derivative **2**; BSA = bis(trimethylsilyl)acetamide.

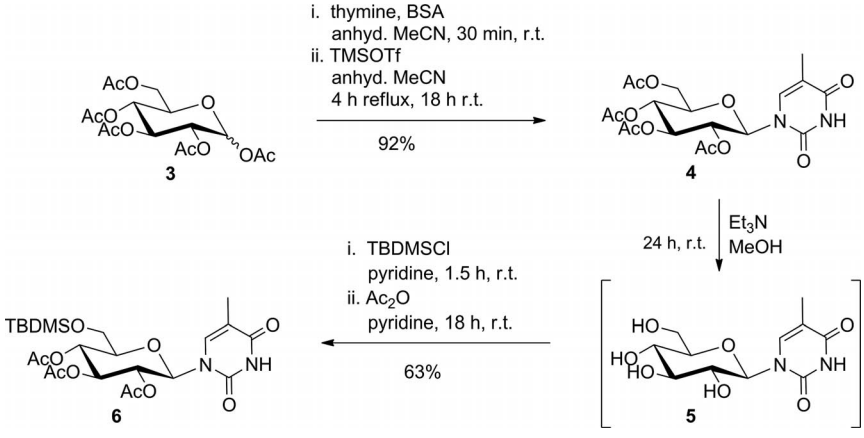
This rather low yield was a result of partial cleavage of the Fmoc-group under the glycosylation conditions. Thus, we chose to first prepare pyranonucleoside **4** starting from peracetylated glycopyranose **3** and then, after complete deacetylation, do the necessary regioselective protecting group reactions to obtain compound **2** in higher quantities.

We first prepared (β-D-glucopyranoside)-thymine (**5**) and (β-D-galactopyranoside)-thymine (**9**). This was again achieved by the Vorbrüggen one-pot method.[15,16] The peracetylated pyranonucleosides were obtained as pure β-anomers in 92% (*gluco* **4**) and 74% (*galacto* **8**), respectively (see Schemes 2 and 3). The lower yield for the *galacto*-configuration is a result of lower anomeric selectivity; the product was formed as an anomeric mixture in a ratio of α/β = 0.1:1. The anomers were separated by column chromatography and mixed fractions were not further purified. Compounds **4** and **8** were then deacetylated in a mixture of methanol and triethylamine to yield desired pyranonucleosides **5** and **9**. Because earlier results showed that the regioselectivity of the reaction of glycopyranoses with FmocCl was rather low whereas that of the reaction with TBDMSCl was very high, pyranonucleosides **5** and **9** were converted into 6′-TBDMS-2′,3′,4′-tri-*O*-acetyl derivatives **6** and **11**. In the case of *gluco*-configuration the same protocol as described previously for the glycopyranoses was employed[14,17] and compound **6** was obtained in a yield of 63% (Scheme 2).



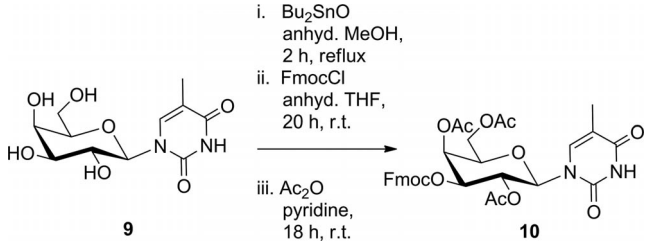
Scheme 3. Synthesis of pyranonucleoside **9**.

In the case of the *galacto*-configured starting material, earlier results indicated that the reaction of derivative **9** with TBDMSCl proceeds with low regioselectivity. Therefore, compound **9** was first reacted with di-*n*-butyltin oxide and then treated with TBDMSCl. It was known for glycosides of D-galactose (e.g. D-lactose) that the reaction with di-*n*-butyltin oxide gave a stannylene acetal, which was then selectively converted into the corresponding *gal*-6-TBDMS-protected derivative.[18,19] This reaction showed a strong dependence on the reagent used. Reaction of the stannylene intermediate with TBDMSCl resulted in the *gal*-6-protected derivative,[19] whereas reaction with an alkyl bromide or tosyl chloride gave the *gal*-3-protected derivative.[19–21] Here, similar results were observed: Reaction of (β-D-galactopyranoside)-thymine (**9**) with di-*n*-butyltin oxide followed by reaction of the intermediate stannylene derivative with TBDMSCl gave 6′-TBDMS-protected derivative **11**, whereas direct reaction of the stannylene intermediate with FmocCl gave 3′-protected derivative **10** (Scheme 4 and Scheme 5). So a detour via the TBDMS-protected pyranonucleoside was necessary. Compound **11** could be obtained in a yield of 47% in addition of a significant amount of

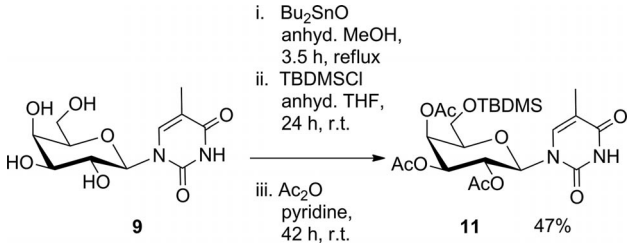


Scheme 2. Synthesis of 6′-TBDMS-protected derivative **6**.

starting material (yield was 80% brsm). The low yield was mainly a result of low conversion of the stannylene intermediate, and further additions of TBDMSCl did not lead to higher conversions.

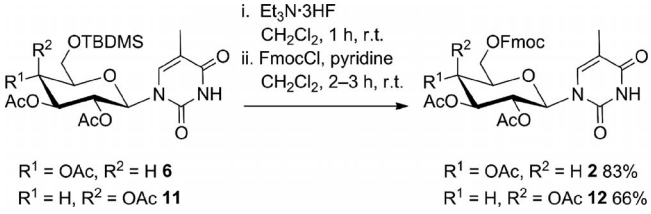


Scheme 4. Dibutylstannylation of **9** followed by reaction with FmocCl.



Scheme 5. Synthesis of 6′-TBDMS-protected derivative **11**.

Compounds **6** and **11** were then converted into corresponding 6′-Fmoc derivatives **2** and **12** in yields of 83% (*gluco*) and 66% (*galacto*), respectively (Scheme 6).



Scheme 6. Synthesis of 6′-Fmoc-protected derivatives **2** and **12**.

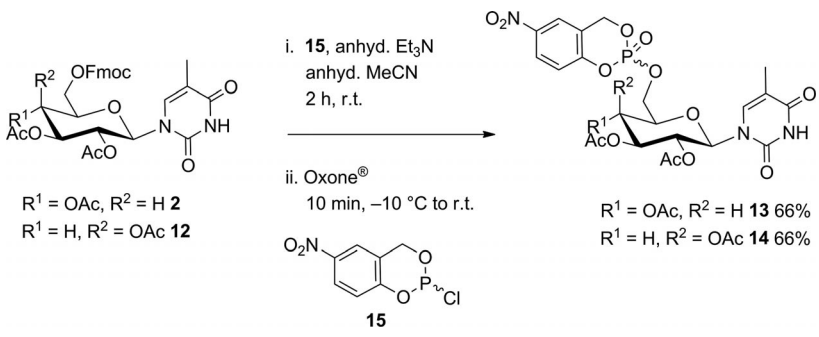
The overall yield for the synthesis of derivative **2** starting from peracetylated sugar **3** by means of this route is 48%, which is clearly better than the 31% yield achieved in the glycosylation reaction with Fmoc-protected precursor **1** (Scheme 1).

Starting from compounds **2** and **12** the *cyclo*Sal-(pyranonucleoside-6′)-phosphates were prepared. As our studies with the glycopyranoses showed, the Fmoc-protected derivatives could very efficiently and selectively be converted into the corresponding *cyclo*Sal-phosphate triesters. A one-pot procedure was needed which involved base-induced elimination of dibenzofulvene and direct reaction of the glycopyranosyl intermediate with 5-NO_2-*cyclo*Saligenylchlorophosphite **15** followed by oxidation.[14] The same procedure was applied to the pyranonucleoside derivatives (Scheme 7). Both 5-NO_2-*cyclo*Sal-[(2′,3′,4′-tri-*O*-acetyl-β-D-glycopyranoside)-thymine-6′]-phosphates **13** and **14** were isolated in yields of 66%.

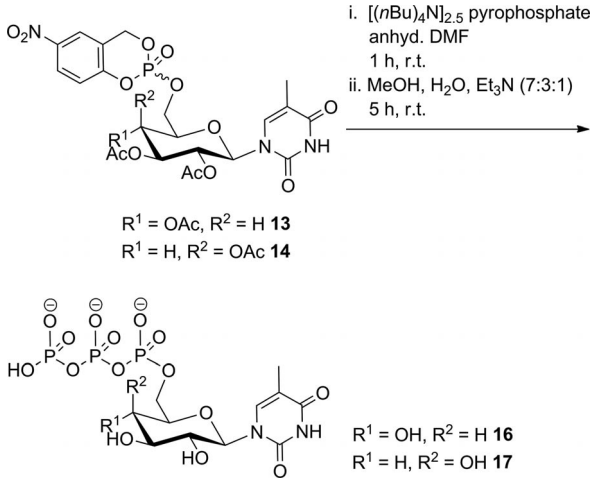
Finally, activated pyranonucleoside-6′-phosphates **13** and **14** were treated with pyrophosphate to give the triphosphates. It was known from earlier studies performed in our laboratories that a lipophilic cation for the pyrophosphate increased its nucleophilicity and solubility in organic solvents to give better results.[8] Thus, 5-NO_2-*cyclo*Sal-[(2′,3′,4′-tri-*O*-acetyl-β-D-glycopyranoside)-thymine-6′]-phosphates **13** and **14** were each reacted with an excess of $[(n\mathrm{Bu_4N})^+]_{2.5}H_{1.5}P_2O_7$ in dry dimethylformamide (DMF) at room temperature.

Very fast conversion of starting material **13** or **14** was observed (monitored by TLC). ^{31}P NMR spectroscopy indicated quantitative conversion of triesters **13** and **14** to corresponding triphosphates **16** and **17**. As expected, isolation of these compounds – especially separation of the excess pyrophosphate – was challenging. Thus, isolation of the triphosphates failed both with reversed phase and size-exclusion chromatography. For this reason we modified the experimental protocol into a "titration-like" method: Triesters **13** and **14** were each dissolved in DMF and small portions of $[(n\mathrm{Bu_4N})^+]_{2.5}H_{1.5}P_2O_7$ in dry DMF were added (Scheme 8).

After addition of the portions, the mixtures were stirred at room temperature for five minutes before analysis by TLC. By the time triesters **13** and **14** were completely consumed water was added and then the solvent was evaporated and deacetylation (methanol, water, triethylamine 7:3:1, room temperature) immediately followed. The total amount of pyrophosphate necessary for complete conversion was 1.1 equiv. Relative to previously described methods, in which up to 10 equiv. of pyrophosphate are used,[5]



Scheme 7. Synthesis of *cyclo*Sal-(pyranonucleoside-6′)-phosphates **13** and **14**.



Scheme 8. Synthesis of pyranonucleoside-6′-triphosphates **16** and **17**.

this is a major advancement. Then, cation exchange to the triethyl ammonium form was performed to facilitate purification on RP-18 silica gel. Triphosphates **16**, **17** were isolated in yields of 63% (**16**) and 72% (**17**).

This "titration-like"-method has an advantage over adding all of the pyrophosphate to the *cyclo*Sal-triester in one portion because the quality of the pyrophosphate can vary especially concerning the counterions (it was not determined whether the exchange of sodium by protons had been achieved completely). For this reason, there is some uncertainty to the exact molar concentration of the pyrophosphate solution. Hence, when adding the pyrophosphate in several portions, only the required amount needs to be added for complete conversion. Furthermore, this experiment provides a new result: an excess of pyrophosphate is not necessary for the reaction to rapidly and efficiently form the triphosphate.

Conclusions

The recently developed route for the synthesis of *cyclo*Sal-(glycopyranosyl-6)-phosphates was successfully applied to the synthesis of such activated phosphate triesters at the 6′-position of pyranonucleosides, as shown for example compounds **13** and **14**. These *cyclo*Sal-triesters could then very efficiently be converted into corresponding pyranonucleoside-6′-triphosphates **16** and **17**. By changing the previously used protocol for the synthesis of triphosphates concerning the order of addition, and the use of as little excess pyrophosphate as possible, purification of the highly polar triphosphates by RP-18 chromatography was simplified to enable isolation in good yields.

Experimental Section

General: Experiments performed in anhydrous solvents were conducted under a nitrogen atmosphere. Acetonitrile was dried by heating to reflux with calcium hydride for several days, followed by distillation and storage with activated molecular sieves. Tetrahydrofuran (THF) was dried by heating to reflux with potassium for several days, followed by distillation and storage with activated molecular sieves. Anhydrous pyridine, DMF and methanol were purchased from Sigma–Aldrich and stored with molecular sieves. Ethyl acetate, petroleum ether, methanol and dichloromethane were distilled before use. Analytical TLC was performed with Macherey–Nagel pre-coated aluminum plates (ALUGRAM Xtra SIL $G/_{UV254}$) with a 0.2 mm layer of silica gel containing a fluorescence indicator; compounds were visualized by staining with 10% H_2SO_4 and heating. NMR spectroscopic data were recorded with Bruker AMX 400 (400 MHz), Bruker DMX 500 (500 MHz) or Bruker AV 600 (600 MHz) spectrometers. All proton and carbon NMR spectroscopy chemical shifts are quoted in ppm and were calibrated by solvent signals where possible. ^{31}P NMR spectroscopy chemical shifts are quoted in ppm by using H_3PO_4 as an external reference. HRMS (ESI) measurements were done with an Agilent Technologies ESITOF 6224 spectrometer.

(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranoside)-thymine (4): The preparation of compound **4** was conducted as described in the literature.[16]

1,2,3,4,6-Penta-*O*-acetyl-D-glucopyranose (**3**, 2.00 g, 5.12 mmol) and thymine (646 mg, 5.12 mmol) were suspended in anhydrous MeCN (30 mL), *N*,*O*-bis(trimethylsilyl)acetamide (2.9 mL, 11.8 mmol) was added. The mixture was stirred at room temperature until a clear solution was obtained. Then, trimethylsilyl triflate (2.3 mL, 12 mmol) was added and the reaction mixture was stirred at reflux temperatures for four hours, followed by 14 h at room temperature. The reaction mixture was diluted with ethyl acetate and washed with water, aq. $NaHCO_3$ solution and brine. The organic layer was dried with sodium sulfate and the solvent was evaporated, followed by column chromatography on silica gel with petroleum ether and an ethyl acetate gradient from 2:3 to 1:2. A yield of 2.14 g (4.69 mmol, 92%) of the product was obtained. R_f (petroleum ether/ethyl acetate, 2:3 v/v): 0.35. ^{1}H NMR: δ = (400 MHz, $CDCl_3$): 8.18 (s, 1 H, NH), 7.13 (d, $^4J_{HH}$ = 1.1 Hz, 1 H, 6-H), 5.85 (d, $^3J_{HH}$ = 9.5 Hz, 1 H, 1′-H), 5.37 (dd, $^3J_{HH}$ = 9.4, $^3J_{HH}$ = 9.4 Hz, 1 H, 3′-H), 5.21–5.12 (m, 2 H, 2′-H, 4′-H), 4.28 (dd, $^2J_{HH}$ = 12.6, $^3J_{HH}$ = 5.1 Hz, 1 H, 6′-H_a), 4.15–4.09 (m, 1 H, 6′-H_b), 3.92 (ddd, $^3J_{HH}$ = 10.1, $^3J_{HH}$ = 5.1, $^4J_{Hthe\ H}$ = 2.1 Hz, 5′-H), 2.10, 2.06, 2.04, 2.00 [s, 3 H, C(O)CH_3], 1.96 (d, $^4J_{HH}$ = 1.0 Hz, 3 H, 7-H_3) ppm. ^{13}C NMR: δ = (101 MHz, $CDCl_3$): 170.5, 169.7, 169.5, 169.4 [*C*(O)CH_3], 162.7 (4), 150.2 (2), 134.5 (6), 112.3 (5), 80.3 (1′), 75.0 (5′), 72.8 (3′), 69.3 (2′), 67.8 (4′), 61.7 (6′), 20.7, 20.5, 20.5, 20.3 [C(O)*C*H_3], 12.6 (7) ppm. HRMS (ESI^+): calcd. for $C_{19}H_{24}N_2O_{11}$ [M + Na^+] 479.1272; found 479.1279. $[\alpha]_D^{20}$ = –14 (c = 0.1, CH_2Cl_2).

(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-galactopyranoside)-thymine (8): The preparation of compound **8** was conducted as described in the literature.[16]

1,2,3,4,6-Penta-*O*-acetyl-D-galactopyranose (**7**; 2.00 g, 5.12 mmol) and thymine (646 mg, 5.12 mmol) were suspended in anhydrous MeCN (30 mL), *N*,*O*-bis(trimethylsilyl)acetamide (2.9 mL, 11.8 mmol) was added. The mixture was stirred at room temperature until a clear solution was obtained. Then, trimethylsilyl triflate (2.3 mL, 12 mmol) was added and the reaction mixture was stirred at reflux temperatures for five hours, followed by 14 h at room temperature. The reaction mixture was diluted with ethyl acetate and washed with water, aq. $NaHCO_3$ solution and brine. The organic layer was dried with sodium sulfate and the solvent was evaporated, followed by column chromatography on silica gel with petroleum ether and an ethyl acetate gradient from 2:3 to 1:2. A yield of 1.72 g (3.77 mmol, 74%) of the product was obtained (before chromatog-

raphy the product was obtained as an anomeric mixture of α/β = 0.1:1, only the β-product was isolated). R_f (petroleum ether/ethyl acetate, 2:3 v/v): 0.35. ^{1}H NMR: δ = (400 MHz, $CDCl_3$): 8.18 (s, 1 H, NH), 7.13 (d, $^4J_{HH}$ = 1.1 Hz, 1 H, 6-H), 5.85 (d, $^3J_{HH}$ = 9.5 Hz, 1 H, 1′-H), 5.37 (dd, $^3J_{HH}$ = 9.4, $^3J_{HH}$ = 9.4 Hz, 1 H, 3′-H), 5.21–5.12 (m, 2 H, 2′-H, 4′-H), 4.28 (dd, $^2J_{HH}$ = 12.6, $^3J_{HH}$ = 5.1 Hz, 1 H, 6′-H_a), 4.15–4.09 (m, 1 H, 6′-H_b), 3.92 (ddd, $^3J_{HH}$ = 10.1, $^3J_{HH}$ = 5.1, $^4J_{HH}$ = 2.1 Hz, 5′-H), 2.10, 2.06, 2.04, 2.00 [s, 3 H, C(O)CH_3], 1.96 (d, $^4J_{HH}$ = 1.0 Hz, 3 H, 7-H_3) ppm. ^{13}C NMR: δ = (101 MHz, $CDCl_3$): 170.5, 169.7, 169.5, 169.4 [*C*(O)CH_3], 162.7 (4), 150.2 (2), 134.5 (6), 112.3 (5), 80.3 (1′), 75.0 (5′), 72.8 (3′), 69.3 (2′), 67.8 (4′), 61.7 (6′), 20.7, 20.5, 20.5, 20.3 [C(O)*C*H_3], 12.6 (7) ppm. HRMS (ESI$^+$): calcd. for $C_{19}H_{24}N_2O_{11}$ [M + Na^+] 479.1272; found 479.1280. $[\alpha]_D^{20}$ = 14 (*c* = 0.1, CH_2Cl_2).

(β-D-Galactopyranoside)-thymine (9): (2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-galactopyranoside)-thymine (**8**; 1.72 g, 3.77 mmol) was suspended in MeOH (14 mL) and triethylamine (4 mL) was added. After stirring at room temperature for 24 h the solvent was evaporated. The product was freeze-dried from water. A yield of 1.09 g (3.77 mmol, 100%) of the product was obtained. ^{1}H NMR: δ = (400 MHz, D_2O): 7.74 (d, $^4J_{HH}$ = 1.0 Hz, 1 H, 6-H), 5.60 (d, $^3J_{HH}$ = 9.2 Hz, 1 H, 1′-H), 4.09 (d, $^3J_{HH}$ = 2.6 Hz, 1 H, 4′-H), 3.98–3.93 (m, 2 H, 2′-H, 5′-H), 3.86 (dd, $^2J_{HH}$ = 9.6, $^3J_{HH}$ = 3.3 Hz, 1 H, 3′-H), 3.81 (m, 2 H, 6′-H), 1.95 (d, $^4J_{HH}$ = 1.0 Hz, 3 H, 7-H_3) ppm. ^{13}C NMR: δ = (101 MHz, D_2O): 166.3 (4), 152.2 (2), 137.4 (6), 112.1 (5), 83.0 (1′), 78.0 (5′), 73.1 (3′), 68.7 (2′), 68.6 (4′), 60.9 (6′), 11.5 (7) ppm. HRMS (ESI$^+$): calcd. for $C_{11}H_{16}N_2O_7$ [M + Na^+] 311.0850; found 311.0848. $[\alpha]_D^{20}$ = 65 (*c* = 0.1, MeOH).

(2′,3′,4′-Tri-*O*-acetyl-6′-*O*-TBDMS-β-D-glucopyranoside)-thymine (6): The preparation of compound **6** was conducted on the basis of a literature-known procedure.[14,17]

Compound **4** (1.44 g, 3.16 mmol) was suspended in MeOH (14 mL) and triethylamine (4 mL) was added. After stirring for 24 h at room temperature the solvent was evaporated and the crude product was dried in vacuo. Then, it was dissolved in anhydrous pyridine (20 mL), TBDMSCl (523 mg, 3.47 mmol) was added and the reaction mixture was stirred for 1.5 h at room temperature. Acetic anhydride (2.9 mL, 31 mmol) was added and stirring at room temperature was continued for 48 h. Then, the solvent was evaporated and after co-evaporation with toluene and dichloromethane the crude product was dissolved in ethyl acetate, washed with water and aq. $NaHCO_3$ solution. The organic layer was dried with sodium sulfate and the solvent was evaporated, followed by column chromatography on silica gel with petroleum ether and an ethyl acetate gradient from 2:1 to 1:1. A yield of 1.05 g (1.99 mmol, 63%) of the product was obtained. R_f (petroleum ether/ethyl acetate, 1:1 v/v): 0.45. ^{1}H NMR: δ = (400 MHz, $CDCl_3$): 8.21 (s, 1 H, NH), 7.13 (d, $^4J_{HH}$ = 0.9 Hz, 1 H, 6-H), 5.81 (d, $^3J_{HH}$ = 9.4 Hz, 1 H, 1′-H), 5.36 (dd, $^3J_{HH}$ = 9.5, $^3J_{HH}$ = 9.5 Hz, 1 H, 3′-H), 5.22 (dd, $^3J_{HH}$ = 9.6, $^3J_{HH}$ = 9.6 Hz, 1 H, 2′-H), 5.12 (dd, $^3J_{HH}$ = 9.5, $^3J_{HH}$ = 9.5 Hz, 1 H, 4′-H), 3.78–3.67 (m, 3 H, 5′-H, 6′-H_2), 2.04, 2.01, 1.99 [s, 3 H, C(O)CH_3], 1.93 (d, $^4J_{HH}$ = 0.65 Hz, 3 H, 7-H_3), 0.88 [s, 9 H, C(CH_3)$_3$], 0.02, 0.01 (s, 3 H, Si-CH_3) ppm. ^{13}C NMR: δ = (101 MHz, $CDCl_3$): 169.9, 169.6, 169.2 [*C*(O)CH_3], 162.8 (4), 150.2 (2), 134.8 (6), 111.9 (5), 80.2 (1′), 77.3 (5′), 73.1 (3′), 69.7 (4′), 67.9 (2′), 61.5 (6′), 25.7 [C(*C*H_3)$_3$], 20.6, 20.6, 20.4 [C(O)*C*H_3], 18.3 [Si-*C*(CH_3)$_3$], 12.4 (7), –5.4 (Si-*C*H_3) ppm. HRMS (ESI$^+$): calcd. for $C_{23}H_{36}N_2O_{10}Si$ [M + H^+] 529.2212; found 529.2215. $[\alpha]_D^{20}$ = 26 (*c* = 0.1, CH_2Cl_2).

(2′,3′,4′-Tri-*O*-acetyl-6′-*O*-TBDMS-β-D-galactopyranoside)-thymine (11): (β-D-galactopyranoside)-thymine (**9**, 270 mg, 0.937 mmol) and di-*n*-butyltin oxide (233 mg, 0.937 mmol) were suspended in anhydrous MeOH (10 mL) and stirred at reflux temperatures for 3.5 h. The solvent was evaporated from the resulting clear solution, the crude product was dried extensively in vacuo. Then, it was dissolved in anhydrous THF and TBDMSCl (141 mg, 0.937 mmol) was added. After stirring for 24 h at room temperature the solvent was evaporated, the residue was dissolved in anhydrous pyridine (5 mL) and acetic anhydride (0.79 mL, 8.4 mmol) was added. After stirring for 42 h at room temperature the solvent was again evaporated and co-evaporated with toluene and dichloromethane. The crude product was dissolved in ethyl acetate and washed with aq. $NaHCO_3$ solution and water. The organic layer was dried with sodium sulfate and the solvent was again evaporated. Column chromatography was performed with silica gel with petroleum ether/ethyl acetate, 1:1 as eluent. A yield of 232 mg (0.439 mmol, 47%; 80% brsm) of the product was obtained. R_f (petroleum ether/ethyl acetate, 1:1 v/v): 0.41. ^{1}H NMR: δ = (400 MHz, $CDCl_3$): 7.97 (s, 1 H, NH), 7.15 (s, 1 H, 6-H), 5.78 (d, $^3J_{HH}$ = 9.2 Hz, 1 H, 1′-H), 5.59 (d, $^3J_{HH}$ = 3.1 Hz, 1 H, 4′-H), 5.29 (dd, $^3J_{HH}$ = 9.8, $^3J_{HH}$ = 9.6 Hz, 1 H, 2′-H), 5.21 (dd, $^3J_{HH}$ = 10.1, $^3J_{HH}$ = 3.2 Hz, 1 H, 3′-H), 3.93 (dd, $^3J_{HH}$ = 6.9, $^3J_{HH}$ = 6.9 Hz, 1 H, 5′-H), 3.72 (dd, $^2J_{HH}$ = 9.9, $^3J_{HH}$ = 5.2 Hz, 1 H, 6′-H_a), 3.61 (dd, $^3J_{HH}$ = 9.6, $^3J_{HH}$ = 8.3 Hz, 1 H, 6′-H_b), 2.20, 2.01, 2.00 [s, 3 H, C(O)CH_3], 1.97 (s, 3 H, 7-H_3), 0.85 [s, 9 H, C(CH_3)$_3$], 0.02, 0.01 (s, 3 H, Si-CH_3) ppm. ^{13}C NMR: δ = (101 MHz, $CDCl_3$): 169.9, 169.7, 169.6 [*C*(O)CH_3], 162.8 (4), 150.1 (2), 134.9 (6), 111.8 (5), 80.8 (1′), 76.2 (5′), 71.2 (3′), 67.4 (2′), 66.7 (4′), 60.2 (6′), 25.7 [C(*C*H_3)$_3$], 20.7, 20.5, 20.5 [C(O)*C*H_3], 18.1 [Si-*C*(CH_3)$_3$], 12.6 (7), –5.6 (Si-*C*H_3) ppm. HRMS (ESI$^+$): calcd. for $C_{23}H_{36}N_2O_{10}Si$ [M + H^+] 529.2212; found 529.2217. $[\alpha]_D^{20}$ = –18 (*c* = 0.1, CH_2Cl_2).

(2′,3′,4′-Tri-*O*-acetyl-6′-*O*-Fmoc-β-D-glucopyranoside)-thymine (2): Route A. The preparation of compound **2** was conducted on the basis of a literature procedure.[16] 1,2,3,4-Tetra-*O*-acetyl-6-*O*-Fmoc-D-glucopyranose (**1**, 200 mg, 0.351 mmol) and thymine (44 mg, 0.351 mmol) were suspended in anhydrous MeCN (8 mL) and *N*,*O*-bis(trimethylsilyl)acetamide (0.200 mL, 0.807 mmol) was added. After the solution became clear, trimethylsilyl triflate (0.16 mL, 0.83 mmol) was added and the reaction mixture was stirred at reflux temperatures for 4.5 h. Then, it was diluted with ethyl acetate and washed with water, aq. $NaHCO_3$ solution and brine. The organic layer was dried with sodium sulfate and the solvent was evaporated, followed by column chromatography on silica gel with petroleum ether/ethyl acetate, 1:1 as eluent. A yield of 70 mg (0.11 mmol, 31%) of the product was obtained.

Route B: The preparation of compound **2** was conducted on the basis of our procedure described in the literature.[14]

(2′,3′,4′-tri-*O*-acetyl-6′-*O*-TBDMS-β-D-glucopyranoside)-thymine (**6**; 500 mg, 0.946 mmol) was dissolved in dichloromethane (3 mL) and triethylamine trihydro fluoride (2.31 mL, 14.2 mmol) was added and the reaction mixture was stirred at room temperature. The course of the reaction was followed by TLC (petroleum ether ether/ethyl acetate, 1:2). After almost all of the starting material has been consumed (one hour), the reaction mixture was diluted with dichloromethane (10 mL) and FmocCl (735 mg, 2.84 mmol) and pyridine (0.69 mL, 8.51 mmol) were added. After stirring for two hours at room temperature the reaction mixture was washed with water, the organic layer was dried with sodium sulfate and the solvent was evaporated. The crude product was purified by column chromatography on silica gel with petroleum ether and an ethyl acetate gradient from 3:1 to 1:2. A yield of 500 mg (0.785 mmol, 83%) of the product was obtained. R_f (petroleum ether/ethyl acetate, 1:2 v/v): 0.56. ^{1}H NMR: δ = (400 MHz, $CDCl_3$): 8.32 (s, 1 H, NH), 7.77 (d, $^3J_{HH}$ = 7.5 Hz, 2 H, $H_{ar,fluorenyl}$), 7.62 (dd, $^3J_{HH}$ =

10.9, $^3J_{HH}$ = 7.5 Hz, 2 H, $H_{ar,fluorenyl}$), 7.44–7.40 (m, 2 H, $H_{ar,fluorenyl}$), 7.35–7.31 (m, 2 H, $H_{ar,fluorenyl}$), 7.13 (d, $^4J_{HH}$ = 1.0 Hz, 1 H, 6-H), 5.87 (d, $^3J_{HH}$ = 9.5 Hz, 1 H, 1′-H), 5.40 (d, $^3J_{HH}$ = 9.5 Hz, 1 H, 3′-H), 5.21–5.16 (m, 2 H, 2′-H, 4′-H), 4.47 (dd, $^3J_{HH}$ = 10.5, $^3J_{HH}$ = 7.2 Hz, 1 H, 6′-H_a), 4.39–4.32 (m, 2 H, fluorenyl-H_2), 4.27–4.24 (m, 2 H, 6′-H_b, fluorenyl-C*H*), 3.96 (ddd, $^3J_{HH}$ = 10.2, $^3J_{HH}$ = 5.1, $^4J_{HH}$ = 2.5 Hz, 5′-H), 2.06, 2.03, 2.00 [s, 3 H, C(O)CH_3]. 1.91 (s, 3 H, 7-H_3) ppm. ^{13}C NMR: δ = (101 MHz, $CDCl_3$): 169.7, 169.5, 169.4 [*C*(O)CH_3], 162.6 (4), 154.7 (fluorenyl-C=O), 150.1 (2), 143.3 ($C_{q,ar}$), 141.3 ($C_{q,ar}$), 134.4 (6), 128.0 (C_{ar}), 127.2 (C_{ar}), 125.1 (C_{ar}), 120.1 (C_{ar}), 112.2 (5), 80.3 (1′) 74.7 (5′), 72.7 (3′), 70.4 (fluorenyl-*C*H_2), 69.3 (4′), 68.0 (2′), 65.3 (6′), 46.7 (fluorenyl-*C*H), 20.5, 20.5, 20.3 [C(O)*C*H_3], 12.5 (7) ppm. HRMS (ESI$^+$): calcd. for $C_{32}H_{32}N_2O_{12}$ [M + H^+] 637.2028; found 637.2034. Calcd. for $C_{32}H_{32}N_2O_{12}$ [M + NH_4^+] 654.2294; found 654.2298. Calcd. for $C_{32}H_{32}N_2O_{12}$ [M + Na^+] 659.1847; found 659.1855. $[a]_D^{20}$ = 7 (*c* = 0.1, CH_2Cl_2).

(2′,3′,4′-Tri-*O*-acetyl-6′-*O*-Fmoc-β-D-galactopyranoside)-thymine (12): The preparation of compound **12** was conducted on the basis of our procedure described in the literature.[14] (2′,3′,4′-tri-*O*-Acetyl-6′-*O*-TBDMS-β-D-galactpyranoside)-thymine (**11**; 364 mg, 0.689 mmol) was dissolved in dichloromethane (3 mL) and triethylamine trihydro fluoride (1.68 mL, 10.3 mmol) was added and the reaction mixture was stirred at room temperature. The course of the reaction was followed by TLC (petroleum ether ether/ethyl acetate, 1:2). After almost all of the starting material was consumed (one hour), the reaction mixture was diluted with dichloromethane (10 mL) and FmocCl (536 mg, 2.07 mmol) and pyridine (0.50 mL, 6.2 mmol) were added. After stirring for three hours at room temperature the reaction mixture was washed with water, the organic layer was dried with sodium sulfate and the solvent was evaporated. The crude product was purified by column chromatography on silica gel with petroleum ether and an ethyl acetate gradient from 3:1 to 1:2. A yield of 291 mg (0.457 mmol, 66%) of the product was obtained. R_f (petroleum ether/ethyl acetate, 1:2 v/v): 0.55. ^{1}H NMR: δ = (400 MHz, $CDCl_3$): 7.97 (s, 1 H, NH), 7.77 (d, $^3J_{HH}$ = 7.6 Hz, 2 H, $H_{ar,fluorenyl}$), 7.59 (dd, $^3J_{HH}$ = 7.1, $^3J_{HH}$ = 4.3 Hz, 2 H, $H_{ar,fluorenyl}$), 7.42 (dd, $^3J_{HH}$ = 7.5, $^3J_{HH}$ = 7.5 Hz, 2 H, $H_{ar,fluorenyl}$), 7.33 (dd, $^3J_{HH}$ = 8.0, $^3J_{HH}$ = 7.6 Hz, 2 H, $H_{ar,fluorenyl}$), 7.15 (s, 1 H, 6-H), 5.83 (d, $^3J_{HH}$ = 9.1 Hz, 1 H, 1′-H), 5.55 (d, $^3J_{HH}$ = 3.0 Hz, 1 H, 4′-H), 5.32 (dd, $^3J_{HH}$ = 9.7, $^3J_{HH}$ = 9.7 Hz, 1 H, 2′-H), 5.22 (dd, $^3J_{HH}$ = 10.1, $^3J_{HH}$ = 3.3 Hz, 1 H, 3′-H), 4.45–4.37 (m, 2 H, fluorenyl-H_2), 4.25–4.23 (m, 3 H, 6′-H_2, fluorenyl-C*H*), 4.19–4.16 (m, 1 H, 5′-H), 2.23 [s, 3 H, C(O)CH_3], 2.02 [s, 6 H, 2 × C(O)CH_3], 1.95 (s, 3 H, 7-H_3) ppm. ^{13}C NMR: δ = (101 MHz, $CDCl_3$): 169.8, 169.8, 169.6 [*C*(O)CH_3], 162.8 (4), 154.6 (fluorenyl-C=O), 150.2 (2), 143.1 ($C_{q,ar}$), 141.3 ($C_{q,ar}$), 134.7 (6), 128.0 (C_{ar}), 127.2 (C_{ar}), 125.2 (C_{ar}), 120.1 (C_{ar}), 112.1 (5), 80.7 (1′), 73.7 (5′), 70.8 (3′), 70.4 (fluorenyl-*C*H_2), 67.1 (2′), 67.0 (4′), 64.9 (6′), 46.6 (fluorenyl-*C*H), 20.7, 20.5, 20.4 [C(O)*C*H_3], 12.6 (7) ppm. HRMS (ESI$^+$): calcd. for $C_{32}H_{32}N_2O_{12}$ [M + H^+] 637.2028; found 637.2031. $[a]_D^{20}$ = 33 (*c* = 0.1, CH_2Cl_2).

5-Nitro-*cyclo*Sal-[(2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranoside)-thymine-6′]-phosphate (13): (2′,3′,4′-Tri-*O*-acetyl-6′-*O*-Fmoc-β-D-glucopyranoside)-thymine (**10**; 0.200 g, 0.314 mmol) was dissolved in anhydrous acetonitrile (3 mL) and 5-nitro-*cyclo*Saligenylchlorophosphite (**15**; 146 mg, 0.628 mmol), dissolved in anhydrous acetonitrile (0.33 mL), was added. After addition of triethylamine (0.260 mL, 1.88 mmol) the reaction mixture was stirred at room temperature. After completion of the reaction (2 h, monitoring by TLC, petroleum ether/ethyl acetate, 1:2) oxone® (965 mg, 1.57 mmol), dissolved in water, was added at 0 °C. The reaction mixture was stirred at room temperature for 10 min, diluted with dichloromethane and washed with water. The organic layer was dried with sodium sulfate and the solvent was evaporated. The crude product was purified by chromatography on silica gel with petroleum ether/ethyl acetate, 1:2 + 2% acetic acid as eluent. A yield of 0.129 g (0.206 mmol, 66%, diastereomeric ratio 1:1.1) of the product was obtained. R_f (petroleum ether/ethyl acetate, 1:2 v/v + 2% HOAc): 0.40. ^{1}H NMR: δ = (400 MHz, $CDCl_3$): 8.32 (s, 2.1 H, N*H*), 8.27–8.23 (m, 2.1 H, $H_{NO2\text{-}ar}$), 8.08 (d, $^4J_{HH}$ = 2.3 Hz, 2.1 H, $H_{NO2\text{-}ar}$), 7.23 (d, $^3J_{HH}$ = 9.0 Hz, 1 H, $H_{NO2\text{-}ar}$), 7.19 (d, $^3J_{HH}$ = 8.9 Hz, 1.1 H, $H_{NO2\text{-}ar}$), 7.15–7.14 (m, 2.1 H, 6-H), 5.86 (d, $^3J_{HH}$ = 9.5 Hz, 1.1 H, 1′-$H_{\beta2}$), 5.86 (d, $^3J_{HH}$ = 9.5 Hz, 1 H, 1′-$H_{\beta1}$), 5.53–5.35 (m, 6.3 H, P-O-CH_2-ar, 3′-H), 5.21–5.10 (m, 4.2 H, 4′-H, 2′-H), 4.46–4.39 (m, 2.1 H, 6′-H_a), 4.35–4.27 (m, 2.1 H, 6′-H_b), 3.98–3.95 (m, 2.1 H, 5′-H), 2.08, 2.01, 1.99 [s, 18.9 H, C(O)CH_3], 1.94, 1.94 (s, 6.3 H, 7-H_3) ppm. ^{13}C NMR: δ = (151 MHz, $CDCl_3$): 169.7, 169.4, 169.3 [*C*(O)CH_3], 162.7 (4), 150.2 (2), 144.1 ($C_{q,NO2\text{-}ar}$), 134.5, 134.4 (6), 125.8, 125.7 ($C_{NO2\text{-}ar}$), 121.7 ($C_{NO2\text{-}ar}$), 119.7 (d, $^4J_{CP}$ = 9.7 Hz, $C_{NO2\text{-}ar}$), 119.7 (d, $^4J_{CP}$ = 9.9 Hz, $C_{NO2\text{-}ar}$), 112.3 (5), 80.3 (1′), 75.2 (d, $^3J_{CP}$ = 6.7 Hz, 5′), 75.1 (d, $^3J_{CP}$ = 6.7 Hz, 5′), 72.7, 72.6 (3′), 69.0, 68.9 (2′), 68.0 (d, $^3J_{CP}$ = 7.7 Hz, P-O-*C*H_2-ar), 67.8 (d, $^3J_{CP}$ = 6.6 Hz, P-O-*C*H_2-ar), 67.5, 67.4 (4′), 66.7 (d, $^3J_{CP}$ = 5.6 Hz, 6′), 66.5 (d, $^3J_{CP}$ = 4.5 Hz, 6′), 20.6, 20.5, 20.3 [C(O)*C*H_3], 12.6 (7) ppm. ^{31}P NMR: δ = (162 MHz, $CDCl_3$): –10.4, –10.7 ppm. HRMS (ESI$^+$): calcd. for $C_{24}H_{26}N_3O_{15}P$ [M + H^+] 628.1174; found 628.1171. Calcd. for $C_{24}H_{26}N_3O_{15}P$ [M + NH_4^+] 645.1440; found 645.1443. Calcd. for $C_{24}H_{26}N_3O_{15}P$ [M + Na^+] 650.0994; found 650.0989.

5-Nitro-*cyclo*Sal-[(2′,3′,4′-tri-*O*-acetyl-β-D-galactopyranoside)-thymine-6′]-phosphate (14): (2′,3′,4′-Tri-*O*-acetyl-6′-*O*-Fmoc-β-D-galactopyranoside)-thymine (**11**; 0.228 g, 0.358 mmol) was dissolved in anhydrous acetonitrile (3 mL) and 5-nitro-*cyclo*Saligenylchlorophosphite (**15**; 167 mg, 0.716 mmol), dissolved in anhydrous acetonitrile (0.54 mL) was added. After addition of triethylamine (0.300 mL, 2.15 mmol) the reaction mixture was stirred at room temperature. After completion of the reaction (2 h, monitoring by TLC, petroleum ether/ethyl acetate, 1:2) oxone® (1.10 g, 1.79 mmol), dissolved in water, was added at 0 °C. The reaction mixture was stirred at room temperature for 10 min, diluted with dichloromethane and washed with water. The organic layer was dried with sodium sulfate and the solvent was evaporated. The crude product was purified by chromatography on silica gel with petroleum ether/ethyl acetate, 1:2 + 2% acetic acid as eluent. A yield of 0.149 g (0.237 mmol, 66%, diastereomeric ratio 1:1.1) of the product was obtained. R_f (petroleum ether/ethyl acetate, 1:2 v/v + 2% HOAc): 0.43. ^{1}H NMR: δ = (400 MHz, $CDCl_3$): 8.60 (s, 2.1 H, N*H*), 8.25–8.23 (m, 2.1 H, $H_{NO2\text{-}ar}$), 8.05 (d, $^4J_{HH}$ = 2.6 Hz, 2.1 H, $H_{NO2\text{-}ar}$), 7.22 (d, $^3J_{HH}$ = 9.0 Hz, 1 H, $H_{NO2\text{-}ar}$), 7.18 (d, $^3J_{HH}$ = 9.0 Hz, 1.1 H, $H_{NO2\text{-}ar}$), 7.15 (d, $^3J_{HH}$ = 1.2 Hz, 2.1 H, 6-H), 5.84 (d, $^3J_{HH}$ = 9.4 Hz, 1 H, 1′-$H_{\beta2}$), 5.82 (d, $^3J_{HH}$ = 9.4 Hz, 1.1 H, 1′-$H_{\beta1}$), 5.53 (d, $^3J_{HH}$ = 2.8 Hz, 1 H, 4′-$H_{\beta1}$), 5.50 (d, $^3J_{HH}$ = 2.5 Hz, 1.1 H, 4′-$H_{\beta2}$), 5.43–5.33 (m, 6.3 H, P-O-CH_2-ar, 2′-H), 5.21–5.16 (m, 2.1 H, 3′-H), 4.41–4.32 (m, 2.1 H, 6′-H_a), 4.30–4.23 (m, 2.1 H, 6′-H_b), 4.23–4.16 (m, 2.1 H, 5′-H), 2.24, 2.18, 2.01, 2.01, 2.00, 1.99, 1.98 [s, 25.2 H, C(O)CH_3, 7-H_3] ppm. ^{13}C NMR: δ = (151 MHz, $CDCl_3$): 169.8, 169.7, 169.7, 169.7, 169.6 [*C*(O)CH_3], 162.9, 162.9 (4), 154.3, 154.3 ($C_{q,NO2\text{-}ar}$), 150.3, 150.3 (2), 144.1 ($C_{q,NO2\text{-}ar}$), 134.7 (6), 125.7 ($C_{NO2\text{-}ar}$), 121.7 ($C_{NO2\text{-}ar}$), 121.4 (d, $^4J_{CP}$ = 9.9 Hz, $C_{NO2\text{-}ar}$), 119.8 (d, $^4J_{CP}$ = 9.5 Hz, $C_{NO2\text{-}ar}$), 119.8 (d, $^4J_{CP}$ = 9.4 Hz, $C_{NO2\text{-}ar}$), 112.2 (5), 80.6, 80.6 (1′), 74.2 (d, $^3J_{CP}$ = 7.7 Hz, 5′), 70.8, 70.8 (3′), 68.1 (d, $^3J_{CP}$ = 6.9 Hz, P-O-*C*H_2-ar), 68.0 (d, $^3J_{CP}$ = 7.2 Hz, P-O-*C*H_2-ar), 66.6 (2′), 66.6 (4′), 65.7 (d, $^3J_{CP}$ = 5.7 Hz, 6′), 65.7 (d, $^3J_{CP}$ = 5.4 Hz, 6′), 20.7, 20.6, 20.5, 20.4 [C(O)*C*H_3], 12.6 (7) ppm. ^{31}P NMR: δ = (162 MHz, $CDCl_3$):

–10.8 ppm. HRMS (ESI$^+$): calcd. for $C_{24}H_{26}N_3O_{15}P$ [M + H$^+$] 628.1174; found 628.1177. Calcd. for $C_{24}H_{26}N_3O_{15}P$ [M + NH_4^+] 645.1440; found 645.1435. Calcd. for $C_{24}H_{26}N_3O_{15}P$ [M + Na$^+$] 650.0994; found 650.0996.

[(*n*Bu$_4$N)$^+$]$_{2.5}$H$_{1.5}$P$_2$O$_7$: Sodium pyrophosphate decahydrate [$Na_4P_2O_7$ 10H_2O] (2.00 g, 4.48 mmol) was dissolved in water and cation exchange was performed with a DOWEX cation exchange resin in its protonated form. After complete elution of the salt, a solution of tetra-*n*-butylammonium hydroxide (40% in water; 7.26 mL, 11.2 mmol) was added. The desired compound was obtained after freeze-drying. ^{31}P-NMR: δ = (162 MHz, D_2O): –9.9 ppm.

(β-D-Glucopyranoside)-thymine-6′-triphosphate (16): 5-Nitro-*cyclo*Sal-[(2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranoside)-thymine-6′]-phosphate (**13**; 20 mg, 0.032 mmol) was dissolved in anhydrous DMF (1 mL). A solution of [(*n*Bu$_4$N)$^+$]$_3$HP$_2$O$_7$ in anhydrous DMF (100 mg/mL) was added in small portions, after every portion the reaction mixture was stirred at room temperature for 5 min and then it was analyzed by TLC (petroleum ether/ethyl acetate, 1:2). After addition of the pyrophosphate solution (0.26 mL, 0.033 mmol, 1.0 equiv., added in five portions) starting material **13** was completely consumed. Water was added to the reaction mixture and then the solvents were evaporated. The residue was taken up in a mixture of methanol, water and triethylamine in a ratio of 7:3:1 (5.5 mL) and stirred for 5 h at room temperature. After evaporation of the solvents cations were exchanged to triethylammonium with a DOWEX® cation exchange resin. After chromatography with RP-18 silica gel with water as eluent, a yield of 16 mg (0.020 mmol, 63%) of the product was obtained. ^{1}H NMR spectroscopy showed that the ratio of triphosphate:triethylammonium was 1:2.8. The missing 0.2 cations were presumed to be sodium. R_f (*i*PrOH/1 M aq. NH_4OAc 2:1): 0.13. ^{1}H NMR: δ = (400 MHz, D_2O): 7.72 (d, $^4J_{HH}$ = 1.0 Hz, 1 H, 6-H), 5.67 (d, $^3J_{HH}$ = 9.2 Hz, 1 H, 1′-H), 4.33–4.25 (m, 2 H, 6′-H), 3.82–3.67 (m, 4 H, 2′-H, 3′-H, 4′-H, 5′-H), 3.24 [q, $^3J_{HH}$ = 7.3 Hz, 16.8 H, $^+$HN(CH_2CH$_3$)$_3$], 1.94 (d, $^4J_{HH}$ = 0.8 Hz, 3 H, 7-H$_3$), 1.31 [t, $^3J_{HH}$ = 7.3 Hz, 25 H, $^+$HN(CH$_2$CH_3)$_3$] ppm. ^{13}C NMR: δ = (151 MHz, D_2O): 166.3 (4), 152.1 (2), 137.5 (6), 112.0 (5), 82.5 (1′), 77.4 (d, $^3J_{CP}$ = 8.7 Hz, 5′), 75.6 (3′), 71.1 (2′), 68.2 (4′), 64.4 ($^2J_{CP}$ = 5.3 Hz, 6′), 46.6 [$^+$HN(CH_2CH$_3$)$_3$], 11.4 (7), 8.2 [$^+$HN(CH$_2$CH_3)$_3$] ppm. ^{31}P NMR: δ = (162 MHz, D_2O): –10.7, –11.1 (d, $^2J_{PP}$ = 19.7 Hz), –23.1 ppm. HRMS (ESI$^-$): calcd. for $C_{11}H_{19}N_2O_{16}P_3$ [M – H$^+$] 526.9875; found 526.9858.

(β-D-Galactopyranoside)-thymine-6′-triphosphate (17): 5-Nitro-*cyclo*Sal-[(2′,3′,4′-tri-*O*-acetyl-β-D-galactopyranoside)-thymine-6′]-phosphate (**14**; 20 mg, 0.032 mmol) was dissolved in anhydrous DMF (1 mL). A solution of [(*n*Bu$_4$N)$^+$]$_3$HP$_2$O$_7$ in anhydrous DMF (100 mg/mL) was added in small portions. After every portion the reaction mixture was stirred at room temperature for 5 min and then it was analyzed by TLC (petroleum ether/ethyl acetate, 1:2). After addition of the pyrophosphate solution (0.28 mL, 0.036 mmol, 1.1 equiv., added in seven portions) starting material **14** was completely consumed. Water was added to the reaction mixture and then the solvents were evaporated. The residue was taken up in a mixture of methanol, water and triethylamine in a ratio of 7:3:1 (5.5 mL) and stirred for 5 h at room temperature. After evaporation of the solvents cations were exchanged to triethylammonium with a DOWEX® cation exchange resin. After chromatography with RP-18 silica gel with water as eluent a yield of 16 mg (0.023 mmol, 72%) of the product was obtained. ^{1}H NMR spectroscopy showed that the ratio of triphosphate:triethylammonium was 1:1.2. The missing 1.8 cations were presumed to be sodium. R_f (*i*PrOH/1 M aq. NH_4OAc 2:1): 0.13. ^{1}H NMR: δ = (400 MHz, D_2O): 7.76 (d, $^4J_{HH}$ = 1.0 Hz, 1 H, 6-H), 5.64 (d, $^3J_{HH}$ = 8.3 Hz, 1 H, 1′-H), 4.31–4.09 (m, 4 H, 4′-H, 5′-H, 6′-H), 3.96–3.89 (m, 2 H, 2′-H, 3′-H), 3.23 [q, $^3J_{HH}$ = 7.3 Hz, 7.2 H, $^+$HN(CH_2CH$_3$)$_3$], 1.95 (s, 3 H, 7-H$_3$), 1.31 [t, $^3J_{HH}$ = 7.3 Hz, 10.8 H, $^+$HN(CH$_2$CH_3)$_3$] ppm. ^{13}C NMR: δ = (151 MHz, D_2O): 166.3 (4), 152.1 (2), 137.5 (6), 112.0 (5), 82.9 (1′), 76.3 (d, $^3J_{CP}$ = 8.8 Hz, 5′), 72.8 (3′), 68.8 (2′), 68.0 (4′), 64.2 (6′), 46.7 [$^+$HN(CH_2CH$_3$)$_3$], 11.4 (7), 8.2 [$^+$HN(CH$_2$CH_3)$_3$] ppm. ^{31}P NMR: δ = (162 MHz, D_2O): –10.6, –11.2 (d, $^2J_{PP}$ = 19.1 Hz), –23.0 ppm. HRMS (ESI$^-$): calcd. for $C_{11}H_{19}N_2O_{16}P_3$ [M – H$^+$] 526.9875; found 526.9933.

Supporting Information (see footnote on the first page of this article): ^{1}H and ^{13}C NMR spectra of compounds **2**, **4**, **6**, **8**, **9**, **11**–**13**, **14**, **16**, and **17**; ^{31}P NMR spectra of compounds **13**, **14**, **16**, and **17**.

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