Chemistry of Natural Compounds and Bioorganic Chemistry

The synthesis of *N*-acetyl-β-L-fucosamine-1-phosphate and uridine 5′-diphospho-*N*-acetyl-β-L-fucosamine

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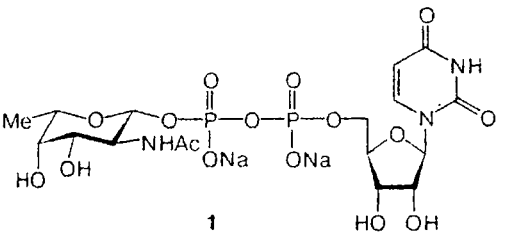
Uridine 5′-(2-acetamido-2,6-dideoxy-β-L-galactopyranosyl) diphosphate (uridine 5′-diphospho-*N*-acetyl-β-L-fucosamine) was synthesized. The key intermediate, 3,4-di-*O*-acetyl-2-azido-2,6-dideoxy-β-L-galactopyranosyl dibenzyl phosphate, was prepared by a previously unknown reaction of cesium dibenzyl phosphate with the corresponding α-glycosyl nitrate and was then converted into the *N*-acetylated glycosyl phosphate and nucleoside diphosphate sugars *via* 3,4-di-*O*-acetyl-2-amino-2,6-dideoxy-β-L-galactopyranosyl phosphate using mild *N*-acetylation and *O*-deacetylation as the last synthetic steps.

Key words: glycosyl phosphates, sugar nucleotides, amino sugars, *N*-acetyl-L-fucosamine, phosphorylation.

2-Acetamido-2,6-dideoxy-L-galactose (*N*-acetyl-L-fucosamine) is a component of many *O*-specific and capsular bacterial polysaccharides, which are characteristic antigens of microorganisms.[1] In particular, the *N*-acetyl-L-fucosamine residue is a part of the capsular polysaccharides from *Staphylococcus aureus* types 5 and 8;[2] more than 70% of strains isolated in staphylococcal infections belong to these types. The nature of the activated precursor of this monosaccharide during biosynthesis of these polymers still remains unclear, although it has been suggested that this role could be played by uridine 5′-(2-acetamido-2,6-dideoxy-β-L-galactopyranosyl) diphosphate (**1**).[3] At present, we are investigating the biosynthesis of capsular polysaccharides from staphylococci. In this communication, we describe the first chemical synthesis of compound **1**. Some results were reported in the preliminary communication.[4]



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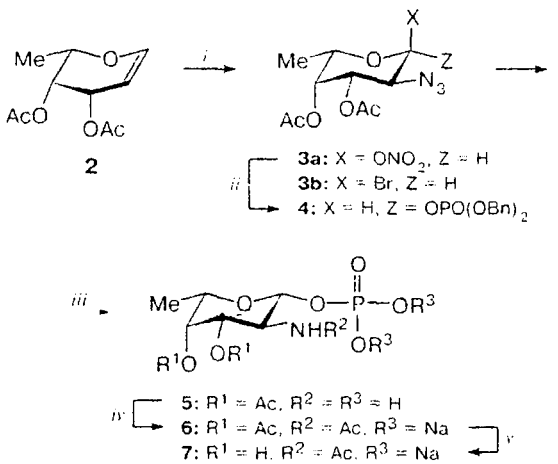
N-Acetyl-L-fucosamine derivatives were synthesized starting from 3,4-di-*O*-acetyl-1,5-anhydro-2,6-dideoxy-L-*lyxo*-hex-1-enitol (3,4-di-*O*-acetyl-L-fucal, **2**), which was prepared by the reaction of 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide with Zn in EtOAc in the presence of *N*-methylimidazole; the procedure was similar to that described[5] for 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide. Azidonitration of fucal **2** carried out as described previously[6] gives rise to 2-azido-3,4-di-*O*-acetyl-2-deoxy-α-L-fucopyranosyl nitrate (**3a**) (Scheme 1), which is easily isolated in the crystalline state. The synthesis of β-phosphate **5** from the products of azidonitration of glycal **2** has been reported recently in relation to the synthesis of guanosine 5′-(2-amino-2,6-dideoxy-β-L-galactopyranosyl) diphosphate.[7] The procedure used in the study cited included reaction of the azidonitration products with LiBr and treatment of the resulting bromide **3b** (the yield was not reported) with dibenzyl phosphate and Ag_2CO_3 to give dibenzyl glycosyl phosphate **4**. Our attempts to employ this procedure to prepare compound **4** were only partly successful; the yield of the target product was ~30%. Therefore, we explored other alternatives for transition from nitrate **3a** to phosphate **5**.

Scheme 1



Reagents and conditions: (*i*) NaN_3, $(NH_4)_2Ce(NO_3)_6$, MeCN (see Ref. 6); (*ii*) $CsOPO(OBn)_2$, DMF; (*iii*) H_2, $Pd(OH)_2/C$, dioxane; (*iv*) NAS, THF–H_2O (1 : 1); (*v*) NaOH, H_2O.

Since nitrate **3a** is converted into bromide **3b** under mild conditions, we suggested that direct transformation of nitrate **3a** into glycosyl phosphate derivatives would also be possible with the use of a sufficiently effective reagent. Cesium dibenzyl phosphate was chosen as the phosphorylating reagent because the use of cesium salts of weak acids in dipolar aprotic solvents (DMF, DMSO) is well known to accelerate S_N2 substitution reactions (so-called "cesium effect"[8]).

Indeed, when compound **3a** was treated with cesium dibenzyl phosphate (1.5 equiv.) in DMF at 20 °C, dibenzyl phosphate **4** was rapidly formed. The reaction was completed in 2 h; the reaction mixture was initially homogeneous and then cesium nitrate precipitated. The target product was obtained in 80% yields after column chromatography. The ^1H, ^{13}C, and ^{31}P NMR spectra of (2-azido-3,4-di-*O*-acetyl-2-deoxy-β-L-fucopyranosyl) dibenzyl phosphate (**4**) confirm the structure assigned to it and correspond to those reported previously.[7]

Compound **4** was converted into 2-amino-3,4-di-*O*-acetyl-2-deoxy-β-L-fucopyranosyl phosphate (**5**) by hydrogenolysis of the benzyl groups accompanied by the simultaneous reduction of the azido group; the best results were obtained when the reaction was carried out in dioxane with $Pd(OH)_2/C$ as the catalyst. Compound **5** was isolated as an inner salt in 97% yield and characterized by NMR spectra (*cf.* Ref. 7).

Transition from the amino sugar **5** to *N*-acetyl derivative **7** requires *N*-acetylation and *O*-deacetylation. Since we intended to use *N*-acetylation for the introduction of the radioactive label into the final product of synthesis, it was necessary to ensure mild reaction conditions ruling out the possibility of O→*N* migration of the acetyl groups.

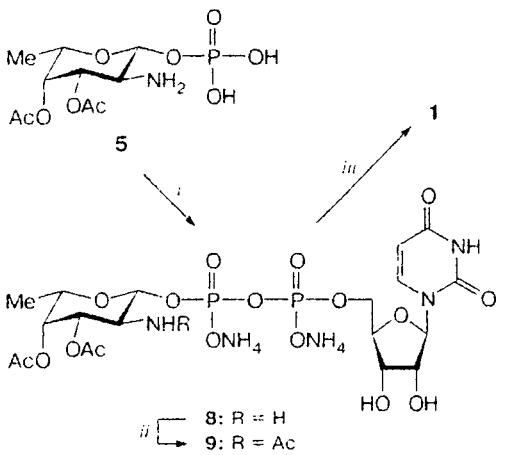
The selective *N*-acetylation of compound **5** was achieved using *N*-acetoxysuccinimide (NAS).[9] Recently,[10] a convenient procedure for the synthesis of this compound has been reported. It is, apparently, suitable for radioactive labeling of the reagent. Compound **5** is smoothly acetylated in an aqueous solution of THF at pH 7.5 (2 h, 20 °C). The ^1H NMR spectrum of product **6** isolated from the reaction mixture after gel chromatography (TSK HW-40, elution by water) exhibits three signals with equal intensities in the region of δ 2.0—2.3, which correspond to the *N*-acetyl and two *O*-acetyl groups.

O-Deacetylation of product **6** (1 h, 20 °C, pH 12) followed by neutralization and separation of the mixture by gel chromatography (TSK HW-40) resulted in the isolation of 2-acetamido-2-deoxy-β-L-fucopyranosyl phosphate (**7**) as the disodium salt in 84% yield. The presence of the signal due to the *N*-acetyl group (δ 2.12) and the signal of H(1) corresponding to the β-configuration of the anomeric center (δ 4.98, t, $J_{1,2} = J_{1,P} = 8.6$ Hz) in the ^1H NMR spectrum confirms the structure of compound **7**.

To convert phosphate **5** into uridine 5′-(2-amino-3,4-di-*O*-acetyl-2-deoxy-β-L-fucopyranosyl) diphosphate (**8**) (Scheme 2), we employed the reaction of glycosyl phosphate with uridine 5′-phosphoimidazolide, prepared *in situ* (*cf.* Ref. 7) by treatment of uridine 5′-monophosphate with *N*,*N*′-carbonyldiimidazole in DMF. The reaction was carried out at a 1.1 : 1 ratio of sugar phosphate **5** to the activated uridine 5′-phosphate derivative; the course of the reaction was monitored by TLC. We developed a convenient procedure for the isolation of product **8** based on gel chromatography with two different columns. Application of the mixture onto a column with Sephadex G-25 and elution with a 25 *mM* solution of NH_4OAc results in the separation of the target product **8** from uridine 5′-monophosphate and P^1,P^2-(uridine-5′) diphosphate, formed as a by-product. Re-chromatography of the major

fraction on a column with TSK HW-40 (elution with water) removes the glycosyl phosphate and NH_4OAc impurities. The yield of compound **8** was 50%. The presence of characteristic ^1H NMR signals with δ 1.02 (d, H(6), J = 6.4 Hz), 1.88, 2.01 (both s, OAc), 3.40 (dd, H(2), $J_{1,2}$ = 8.6 Hz, $J_{2,3}$ = 10.8 Hz), and 5.20 (t, H(1), $J_{1,P}$ = 8.6 Hz) corresponding to β-fucosamine and signals with δ 5.78 (m), 7.72 (d, J = 8.3 Hz) of the uridine residue, as well as two doublets with δ −10.3 (J = 19.3 Hz) and −13.0 (J = 19.3 Hz) in the ^{31}P NMR spectrum, confirms the structure of diphosphate **8**.

Scheme 2



Reagents and conditions: (*i*) uridine 5′-phosphoimidazolide, DMF; (*ii*) NAS, THF—H_2O (1 : 1); (*iii*) NaOH, H_2O.

Compound **1** was prepared by selective *N*-acetylation of sugar nucleotide diphosphate **8** by the method described above using 3 equiv. of NAS (the reaction is complete in 5 h). An aliquot of the reaction mixture was separated on a column with TSK HW-40; the completeness of the reaction was confirmed by the ^1H NMR spectrum of the product **9** thus isolated, which contained three equally intense signals in the region of δ 2.0—2.3. The product was *O*-deacetylated at pH 12 for 3 h. After neutralization of the reaction mixture, compound **1** was isolated by gel chromatography on a column with TSK HW-40. The yield of uridine 5′-(2-acetamido-2-deoxy-β-L-fucopyranosyl) diphosphate (**1**) isolated as the disodium salt, homogeneous according to the data of paper electrophoresis, amounted to 97%; its structure was confirmed by ^1H, ^{13}C, and ^{31}P NMR spectra. In particular, the presence of ^1H NMR signals of the uridine residue at δ 7.75 (d, H(6)) and δ 5.78 (m, H(5), H(1′)) and signals at δ 4.79 (t, H(1″), $J_{1,2}$ = $J_{1,P}$ = 8.6 Hz), δ 1.85 (NHAc), and δ 1.06 (d, H(6″), $J_{5,6}$ = 6.9 Hz), corresponding to the 2-acetamido-2-deoxy-β-L-fucopyranose residue, and two ^{31}P NMR doublets proves the structure of diphosphate **1**.

The synthetic scheme for the preparation of uridine 5′-(2-acetamido-2-deoxy-β-L-fucopyranosyl diphosphate) (**1**) and 2-acetamido-2-deoxy-β-L-fucopyranosyl phosphate (**7**) with *N*-acetylation as the final stage, developed in this study, appears the most convenient for the introduction of a radioactive label from [^{14}C]acetate into these compounds needed for biosynthetic studies.

Experimental

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or D_2O at 300 K on a Bruker WM-250 spectrometer using acetone as the internal standard. ^{31}P NMR spectra were measured in $CDCl_3$ or D_2O at 300 K on a Bruker AM-200 spectrometer with 85% H_3PO_4 as the external standard. UV spectra were recorded on a Specord UV—VIS instrument (GDR). Electrophoresis was performed on a PVEF-1 instrument at a voltage gradient of 10 V cm^{-1} in a 0.05 *M* triethylammonium bicarbonate buffer (pH 7.5) on Filtrak FN-16 paper; the phosphate was detected by the reagent described previously.[11] Thin layer chromatography was carried out on glass plates with a fixed silica gel (Merck) layer, while silica gel LiChroprep Si 60 (Merck) was used for column chromatography. Gel chromatography was carried out on Sephadex G-25 superfine (600×25 mm) and TSK HW-40(S) (800×20 mm) columns. The separation was monitored using a Knauer differential refractometer. Solvents: DMF was distilled from CaH_2 *in vacuo* and stored over 4 Å molecular sieves; MeCN and EtOAc were distilled from P_2O_5 and stored over 4 Å molecular sieves; dioxane was distilled from $LiAlH_4$ immediately prior to use; MeOH was distilled from $Mg(OMe)_2$ by the standard procedure; and THF was distilled from Na—Ph_2CO under Ar. Reagents from Sigma were used. 1-Methylimidazole and *N*,*N*′-carbonyldiimidazole (Fluka) and $Pd(OH)_2$/C (Aldrich) were used as received. Cesium dibenzyl phosphate was prepared by mixing dibenzyl hydrogen phosphate with 1.01 equiv. of Cs_2CO_3 in acetonitrile. The solvent was distilled off and the residue was dried *in vacuo* at 25 °C.

3,4-Di-*O*-acetyl-1,5-anhydro-2,6-dideoxy-L-*lyxo*-hex-1-enitol (2). 2,3,4-Tri-*O*-acetyl-α-L-fucopyranosyl bromide[7] (5.2 g, 14.7 mmol) in 15 mL of EtOAc was added with stirring over a period of 15 min to a mixture of Zn powder (5.7 g) and 1-methylimidazole (1.17 mL) in 77 mL of boiling anhydrous EtOAc. The mixture was refluxed for 20 min and cooled to 20 °C. The reaction mixture was filtered through celite and the filtrate was washed with 10% HCl (2×15 mL), a saturated solution of $NaHCO_3$ (3×15 mL), and water and dried with Na_2SO_4. The organic phase was concentrated *in vacuo* to give 2.21 g (70%) of compound **2**, which was then used to prepare **3a**[6] without additional purification. ^1H NMR ($CDCl_3$), δ: 1.22 (d, 3 H, H(6), $J_{5,6}$ = 6.7 Hz); 1.96 (s, 3 H, OAc); 2.10 (s, 3 H, OAc); 4.20 (br.q, 1 H, H(5)); 4.62 (dt, 1 H, H(3), $J_{2,3}$ = $J_{1,3}$ = 6.0 Hz, $J_{3,4}$ = 1.2 Hz); 5.24 (br.d, 1 H, H(2)); 5.56 (br.d, 1 H, H(4)); 6.45 (dd, 1 H, H(1), $J_{1,2}$ = 1.0 Hz) (*cf.* Ref. 7).

2-Azido-3,4-di-*O*-acetyl-2-deoxy-β-L-fucopyranosyl dibenzyl phosphate (4). Cesium dibenzyl phosphate (480 mg, 1.12 mmol) was added at 20 °C with stirring to a solution of compound **3a**[6] (250 mg, 0.78 mmol) in 0.5 mL of anhydrous DMF. The mixture was stirred for an additional 2 h, poured into 3 mL of brine, and extracted with $CHCl_3$ (3×3 mL). The organic phase was washed with water, dried with Na_2SO_4, concentrated *in vacuo*, and chromatographed in the petroleum ether—EtOAc system to give 332 mg (80%) of compound **4**, a syrup, R_f 0.27 (petroleum ether—EtOAc, 2 : 1). ^1H NMR ($CDCl_3$), δ: 1.18 (d, 3 H, H(6), $J_{5,6}$ = 6.9 Hz); 2.03 (s, 3 H, OAc); 2.15 (s, 3 H, OAc); 3.79 (dd, 1 H, H(2), $J_{1,2}$ = 8.6 Hz, $J_{2,3}$ = 10.4 Hz); 3.82 (q, 1 H, H(5)); 4.82 (dd, 1 H, H(3), $J_{3,4}$ = 4.1 Hz); 5.09 (m, 5 H, H(1), $PhCH_2$); 5.18 (br.d, 1 H, H(4)); 7.31 (m, 10 H, Ph). ^{13}C NMR ($CDCl_3$), δ: 15.6 (C(6)); 20.5 ($\underline{C}H_3CO$); 60.8

(d, C(2), J = 9.6 Hz); 68.7; 69.0 (d, $PhCH_2$, J = 4.1 Hz); 69.1 (d, $PhCH_2$, J = 4.3 Hz); 70.1; 71.4; 97.5 (d, C(1), J = 5.5 Hz); 127.7, 127.8, 128.5, 135.2, 135.3, 135.5 (all Ph); 169.6, 170.2 (both $COCH_3$). ^{31}P NMR ($CDCl_3$), δ: −2.75.

2-Amino-3,4-di-*O*-acetyl-2-deoxy-β-L-fucopyranosyl phosphate (5). Freshly prepared compound **4** (250 mg, 0.47 mmol) was dissolved in 3 mL of dry dioxane and hydrogenated under atmospheric pressure for 8 h and 25 °C over 40 mg of the 10% $Pd(OH)_2/C$ catalyst. The catalyst was filtered off and washed with dioxane (3×5 mL). Lyophilization of the dioxane solution gave 134 mg (97%) of compound **5**, R_f 0.35 (propan-2-ol—1 M aqueous NH_4OAc, 2 : 1). ^1H NMR (D_2O), δ: 1.23 (d, 3 H, H(6), $J_{5,6}$ = 6.9 Hz); 2.09 (s, 3 H, OAc); 2.21 (s, 3 H, OAc); 3.59 (br.t, 1 H, H(2), $J_{2,3}$ = 10.4 Hz); 4.13 (br.q, 1 H, H(5)); 5.28 (m, 3 H, H(1), H(3), H(4)). ^{13}C NMR (D_2O), δ, $J_{C,P}$/Hz: 15.2 (C(6)); 20.1, 20.4 (both signals CH_3CO); 51.9 (d, C(2), J = 9.5 Hz); 69.6; 70.1; 70.5; 94.2 (d, C(1), J = 4.8 Hz); 171.0, 173.6 (both signals $COCH_3$). ^{31}P NMR ($CDCl_3$), δ: −1.56.

Disodium 2-acetamido-2-deoxy-β-L-fucopyranosyl phosphate (7). A solution of *N*-acetoxysuccinimide (NAS)[10] (42 mg, 0.28 mmol) in 0.44 mL of aqueous THF (1 : 1) was added with stirring at 20 °C to a solution of compound **5** (40 mg, 0.14 mmol) in 0.22 mL of water. Stirring was continued for 2 h; the pH of the reaction mixture equal to 7.5 was maintained by adding 0.1 M NaOH. The reaction mixture was cooled to 0 °C, 1 M NaOH (0.1 mL) was added, and the mixture was kept for 1 h at 20 °C and neutralized by the Dowex 50W×8 cation exchanger (H^+) to pH 7.5 using a pH-meter. The cation exchanger was filtered off and washed with water (3×2 mL). The filtrate was concentrated *in vacuo* to 0.1 mL and applied onto a TSK HW-40 column, and the column was washed with water. The fractions containing the product were combined, concentrated *in vacuo* to 5 mL, and treated with the Dowex 50W×8 cation exchange resin (Na^+, 0.5 mL). The cation exchange resin was filtered off and washed with water (3×5 mL). Lyophilization from water gave 34 mg (84%) of compound **7**, $[\alpha]_D^{25}$ −4.50° (c 1.6, H_2O). ^1H NMR (D_2O), δ: 1.35 (d, 3 H, H(6), $J_{5,6}$ = 6.9 Hz); 2.12 (s, 3 H, NAc); 3.27 (br.t, 1 H, H(2), $J_{2,3}$ = 10.4 Hz); 3.89 (m, 3 H, H(3), H(4), H(5)); 4.98 (t, 1 H, H(1), $J_{1,2} = J_{1,P}$ = 8.6 Hz).

Diammonium uridine 5′-(2-amino-3,4-di-*O*-acetyl-2-deoxy-β-L-fucopyranosyl) diphosphate (8). Tri(*n*-butyl)amine (0.073 mL, 0.308 mmol) was added with stirring at 20 °C to a solution of uridine 5′-phosphate (100 mg, 0.308 mmol) in anhydrous dioxane. The reaction mixture was stirred for an additional 15 min and lyophilized and the residue was dissolved in 0.5 mL of anhydrous DMF. *N,N′*-Carbonyldiimidazole (150 mg, 0.92 mmol) was added and the mixture was stirred under Ar for 3 h at 20 °C. Then anhydrous MeOH (0.035 mL) was added and the mixture was stirred for 20 min and kept *in vacuo* (1 Torr, 20 °C) for 15 min to remove unreacted MeOH. Compound **5** (100 mg, 0.339 mmol) in 0.3 mL of anhydrous DMF was added at 20 °C to the resulting solution of uridine 5′-phosphoimidazolide. The mixture was stirred under Ar for 24 h, diluted with 3 mL of a 25 mM aqueous solution of NH_4OAc, and washed with $CHCl_3$ (1×2 mL). The aqueous layer was concentrated *in vacuo* to 0.5 mL and applied onto a column with Sephadex G-25. The column was washed with a 25 mM aqueous solution of NH_4OAc, the separation being monitored using a UV detector (254 nm). The fractions containing the product were concentrated *in vacuo* to 1.5 mL, the solution was applied onto a column with TSK HW-40, and the column was washed with water. Lyophilization of the fractions containing the product gave 96 mg (50%) of compound **8**; paper electrophoresis $M_{picrate}$ 1.36. ^1H NMR (D_2O), δ: 1.06 (d, 3 H, H(6″), $J_{5'',6''}$ = 6.9 Hz); 1.88 (s, 3 H, OAc); 2.01 (s, 3 H, OAc); 3.40 (dd, 1 H, H(2″), $J_{2'',3''}$ = 10.8 Hz); 4.10 (m, 5 H, H(2′), H(3′), H(4′), H(5′), H(5″)); 5.08 (m, 2 H, H(3″), H(4″)); 5.20 (t, 1 H, H(1″), $J_{1'',2''} = J_{1'',P}$ = 8.6 Hz); 5.78 (m, 2 H, H(5), H(1′)); 7.73 (d, 1 H, H(6), $J_{5,6}$ = 8.3 Hz). ^{13}C NMR (D_2O), δ: 15.6 (C(6″)); 20.5, 20.6 (both signals $COCH_3$); 52.1 (d, C(2″), J = 8.9 Hz); 55.7 (d, C(5′), J = 5.4 Hz); 69.9; 70.2; 70.7; 70.9; (C(3″), C(4″), C(5″), C(2′)); 74.4 (C(3′)); 83.6 (d, C(4′), J = 8.8 Hz); 89.1 (C(1′)); 95.1 (d, C(1″), J = 3.8 Hz); 103.2 (C(5)); 142.2 (C(6)); 156.0 (C(2)); 166.7 (C(4)); 173.1, 174.1 (both signals $COCH_3$). ^{31}P NMR (D_2O), δ: −10.3 (d, J = 19.3 Hz); −13.0 (d, J = 19.3 Hz).

Disodium uridine 5′-(2-acetamido-2-deoxy-β-L-fucopyranosyl) diphosphate (1). *N*-acetoxysuccinimide (22.4 mg, 0.12 mmol) in 0.4 mL of aqueous THF (1 : 1) was added at 20 °C to a solution of compound **8** (24 mg, 0.038 mmol) in 0.3 mL of water. The reaction mixture was stirred for 5 h; the pH value equal to 7.5 was maintained by adding 0.1 M NaOH. The mixture was cooled to 0 °C, 1 M NaOH (0.2 mL) was added, and the mixture was kept for 3 h at 20 °C and neutralized by the Dowex 50W×8 (H^+) cation exchanger to pH 7.5 using a pH-meter. The cation exchanger was filtered off and washed with water (3×3 mL) and the filtrate was concentrated *in vacuo* to 0.2 mL and subjected to gel chromatography on a column with TSK HW-40, the product being eluted with water. The fractions containing the product were lyophilized, yield 23 mg (97%) (the content of compound **1**, determined from absorption at 262 nm, was 96%). UV, λ_{max} 262 nm. ^1H NMR (D_2O), δ: 1.06 (d, 3 H, H(6″), $J_{5'',6''}$ = 6.9 Hz); 1.85 (s, 3 H, NAc); 3.66 (m, 2 H, H(3″), H(4″)); 3.72 (dd, 1 H, H(2″), $J_{1'',2''}$ = 8.6 Hz, $J_{2'',3''}$ = 10.8 Hz); 4.10 (m, 6 H, H(2′), H(3′), H(4′), H(5′), H(5″)); 4.79 (t, 1 H, H(1″), $J_{1'',P}$ = 8.6 Hz); 5.78 (m, 2 H, H(5), H(1′)); 7.75 (d, 1 H, $J_{5,6}$ = 8.3 Hz). ^{13}C NMR (D_2O), δ: 15.3 (C(6″)); 22.2 ($COCH_3$); 52.1 (d, C(2″), J = 8.9 Hz); 64.7 (d, C(5′), J = 5.4 Hz); 69.4; 70.2, 70.9, 71.2 (C(3″), C(4″), C(5″), C(2′)); 73.5 (C(3′)); 83.0 (d, C(4′), J = 8.8 Hz); 88.1 (C(1′)); 96.1 (C(1″), J = 3.8 Hz); 102.4 (C(5)); 141.4 (C(6)); 151.5 (C(2)); 165.8 (C(4)); 173.1 ($COCH_3$). ^{31}P NMR (D_2O), δ: −10.1 (d, J = 19.3 Hz); −12.5 (d, J = 19.3 Hz).

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