

## Chemistry of Natural Compounds, Bioorganic, and Biomolecular Chemistry

### A novel synthesis of *N*-acetyl- $\alpha$ -D-fucosamine 1-phosphate and uridine 5'-diphospho-*N*-acetyl- $\alpha$ -D-fucosamine

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In connection with studies on the biosynthesis of capsular polysaccharides from *Staphylococcus aureus*, a new synthesis of uridine 5'-(2-acetamido-2,6-dideoxy- $\alpha$ -D-galactopyranosyl diphosphate) (uridine 5'-diphospho-*N*-acetyl- $\alpha$ -D-fucosamine) using 2-azido-3,4-di-*O*-acetyl-2,6-dideoxy- $\alpha$ -D-galactopyranosyl nitrate as the key intermediate was carried out. The reaction of this product with cesium dibenzyl phosphate smoothly affords the corresponding  $\beta$ -glycosyl dibenzyl phosphate, which undergoes anomerization on treatment with  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  and 2-bromopyridine to give  $\alpha$ -glycosyl dibenzyl phosphate in high yield. This product was then transformed into 2-amino-3,4-di-*O*-acetyl-2,6-dideoxy- $\alpha$ -D-galactopyranosyl phosphate, subsequently converted into 2-acetamido-2,6-dideoxy- $\alpha$ -D-galactopyranosyl phosphate and the target nucleoside diphosphate sugar.

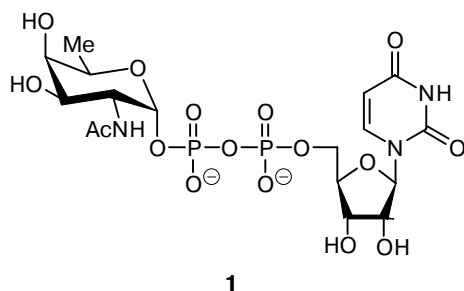
**Key words:** glycosyl phosphates, sugar nucleotides, amino sugars, *N*-acetyl-D-fucosamine, phosphorylation.

Study of the biosynthesis of capsular polysaccharides of staphylococci is of substantial interest as regards the search for new ways for combating the staphylococcal infection. In this respect, polysaccharides from *Staphylococcus aureus* serotypes 5 and 8 built of *N*-acetyl-D-mannosaminuronic acid, 2-acetamido-2,6-dideoxy-L-galactose (*N*-acetyl-L-fucosamine), and 2-acetamido-2,6-dideoxy-D-galactose (*N*-acetyl-D-fucosamine) residues<sup>1</sup> are especially important; more than 70% of

clinical isolates of staphylococci belong to these serotypes.

The genetic data on the biosynthesis of these polymers<sup>2</sup> suggest that the polysaccharide chains are formed according to a block mechanism, *i.e.*, through assembling of trisaccharide repeating units on a polyprenyl phosphate acceptor. The *N*-acetyl-D-fucosamine residue whose activated form is uridine 5'-(2-acetamido-2,6-dideoxy- $\alpha$ -D-galactopyranosyl diphosphate)

(1) acts as the monosaccharide initiating the chain growth.



Previously, this compound was synthesized by our research group<sup>3</sup> in connection with studies<sup>4</sup> of the biosynthesis of teichoic acid in *Streptomyces chrysomallus*. *N*-Acetyl-D-fucosamine tri-*O*-acetate served as the starting material in this synthesis. Since the introduction of a radioactive label into compound 1 presents substantial interest for biochemical research, it was necessary to develop a synthetic scheme that would permit introduction of [<sup>14</sup>C]-acetate at final steps of the synthesis. To this end, we investigated an approach that we have developed previously and that proved useful in the synthesis of another hypothetical substrate of the biosynthesis of capsular polysaccharides from staphylococci, namely, uridine 5'-(2-acetamido-2,6-dideoxy-β-L-galactopyranosyl diphosphate).<sup>5</sup> This approach is based on the use of 2-azido-2,6-dideoxy sugar derivatives and a new reaction for the preparation of glycosyl phosphates from glycosyl nitrates.<sup>6,7</sup>

## Results and Discussion

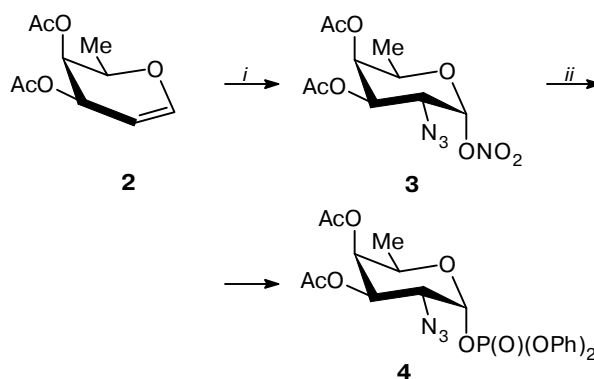
The synthesis of *N*-acetyl-D-fucosamine derivatives described in this work makes use of 3,4-di-*O*-acetyl-1,5-anhydro-2,6-dideoxy-D-*lyxo*-hex-1-enitol (3,4-di-*O*-acetyl-D-fucal, 2) as the initial compound. This was prepared by the reaction of 2,3,4-tri-*O*-acetyl-α-D-fucopyranosyl bromide<sup>8</sup> with Zn in EtOAc in the presence of *N*-methylimidazole,<sup>9</sup> similarly to the procedure that we have used<sup>5</sup> in the synthesis of its L-enantiomer.

Azidonitration of D-fucal 2 gives rise to 2-azido-3,4-di-*O*-acetyl-2-deoxy-α-D-fucopyranosyl nitrate (3) (Scheme 1); it is carried out similarly to the synthesis of the previously described<sup>10</sup> L-isomer of compound 3.

The presence of characteristic absorption bands of the —ONO<sub>2</sub> ( $\nu_{\max}$  = 1650 cm<sup>-1</sup>) and —N<sub>3</sub> ( $\nu_{\max}$  = 2115 cm<sup>-1</sup>) groups in the IR spectrum of compound 3, the coincidence of its <sup>1</sup>H NMR spectrum with that of the L-isomer, the value of specific optical rotation, which is close in magnitude but opposite in sign to that for the L-isomer (*cf.* Ref. 10), and the data from the <sup>13</sup>C NMR spectrum confirm the structure of the azidoglycosyl nitrate 3.

α-Glycosyl phosphate 4 was prepared using a reaction that we have developed recently,<sup>6,7</sup> namely, treat-

Scheme 1



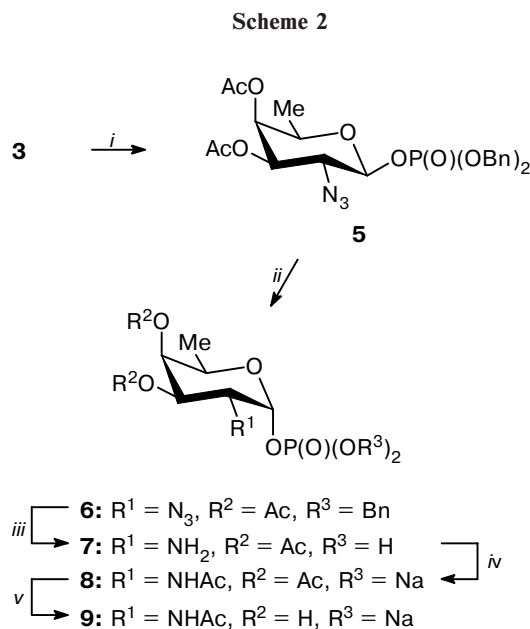
**Reagents and conditions:** *i*) NaN<sub>3</sub>, Ce(NH<sub>4</sub>)<sub>2</sub>(NO<sub>3</sub>)<sub>6</sub>, MeCN; *ii*) CsOP(O)(Ph)<sub>2</sub>, 18-crown-6, DMF.

ment of α-glycosyl nitrate 3 with CsOP(O)(Ph)<sub>2</sub> in DMF. At 20 °C (5 h), this reaction, like the reaction with L-fucose derivatives, smoothly results in the formation of thermodynamically stable α-glycosyl diphenyl phosphate 4, which was isolated in 78% yield. The structure of this compound is confirmed by the signals for H(1) (δ 6.08, dd,  $J_{1,2}$  = 3.4 Hz,  $J_{1,P}$  = 6.4 Hz) and H-2 (δ 3.62, ddd,  $J_{2,3}$  = 11.1 Hz,  $J_{2,P}$  = 3.1 Hz) in the <sup>1</sup>H NMR spectrum and by a single signal observed in the <sup>31</sup>P NMR spectrum (δ -12.7).

However, the attempts to pass from compound 4 to glycosyl phosphate using the standard procedure<sup>11</sup> of removal of the phenyl groups by hydrogenolysis over PtO<sub>2</sub> proved unsuccessful. During hydrogenolysis of compound 4 in dioxane, hydrogen absorption was sharply retarded after the fast reduction of the azido group to the amino group; instead of the removal of phenyl groups, only slow cleavage of the glycosyl phosphate bond in the phosphoric triester was observed giving rise to 2-amino-3,4-di-*O*-acetyl-2,6-dideoxy-D-galactose. The difficulty of hydrogenolysis of the phenyl groups in the peptidic phosphoric triesters containing an amino group has been noted previously.<sup>12</sup> Although successful transformation of α-glycosyl diphenyl phosphate derivatives of 2-amino-2-deoxy-D-glucose hydrochloride<sup>13</sup> and 3-azido-3-deoxy-D-glucose<sup>14</sup> into the corresponding glycosyl phosphates has been described in the literature, it is necessary to carry out hydrogenolysis in an acidic medium to ensure complete removal of the phenyl groups. In our case, these conditions are inapplicable because of the higher sensitivity of 6-deoxyhexose derivatives to acids.

In the synthesis of L-fucosamine-1-phosphate derivative described previously,<sup>5</sup> hydrogenolysis of the benzyl protective groups was successfully accomplished over a palladium catalyst; therefore, we decided to study another approach to the synthesis of the target α-glycosyl phosphate. This approach is based on the transformation of compound 3 into β-glycosyl dibenzyl phosphate 5, containing benzyl protective group at the phosphate

residue followed by anomerization of the product into thermodynamically more stable  $\alpha$ -anomer **6** (Scheme 2).



**Reagents and conditions:** (i) CsOPO(OBn)<sub>2</sub>, DMF; (ii) BF<sub>3</sub> · Et<sub>2</sub>O, 2-bromopyridine, CH<sub>2</sub>Cl<sub>2</sub>; (iii) H<sub>2</sub>, Pd(OH)<sub>2</sub>/C, dioxane; (iv) *N*-acetoxysuccinimide (NAS), THF–H<sub>2</sub>O (1:1); (v) NaOH, H<sub>2</sub>O.

Treatment of compound **3** with cesium dibenzyl phosphate in DMF at 20 °C results in the fast (2 h) formation of  $\beta$ -glycosyl dibenzyl phosphate **5**, which was isolated in 79% yield. The <sup>1</sup>H, <sup>13</sup>C, and <sup>31</sup>P NMR spectra of the resulting phosphoric triester confirm its structure and correspond to the spectra of the L-isomer of this compound described previously.<sup>5</sup>

The attempts to induce anomerization of compound **5** into thermodynamically more stable  $\alpha$ -glycosyl dibenzyl phosphate **6** by treatment with hydrogen dibenzyl phosphate yielded a mixture of anomeric phosphoric triesters; however, under various experimental conditions, we were unable to attain complete anomerization. This problem was solved only by using BF<sub>3</sub> · Et<sub>2</sub>O as the acid catalyst in the presence of weak pyridine bases (these reagents have recently been proposed for anomerization of dibenzyl phosphates of benzylated hexoses<sup>15</sup>). Treatment of  $\beta$ -glycosyl dibenzyl phosphate **5** with boron trifluoride etherate (0.5 equiv.) and 2-bromopyridine (2 equiv.) in CH<sub>2</sub>Cl<sub>2</sub> for 1 h at 20 °C furnished  $\alpha$ -glycosyl dibenzyl phosphate **6** in 72% yield (after column chromatography). The data from <sup>1</sup>H NMR spectroscopy ( $\delta$ : 5.95 (dd, H(1),  $J_{1,2} = 3.4$  Hz,  $J_{1,P} = 6.3$  Hz); 3.60 (ddd, H(2),  $J_{2,3} = 11.0$  Hz,  $J_{2,P} = 3.1$  Hz); 96.1 (d, C(1),  $J_{C,P} = 5.6$  Hz); 57.3 (d, C(2),  $J_{C,P} = 8.6$  Hz)) imply the  $\alpha$ -configuration at the C(1) atom and confirm completely the structure of compound **6**. It should be noted that under conditions described previously<sup>15</sup> (the

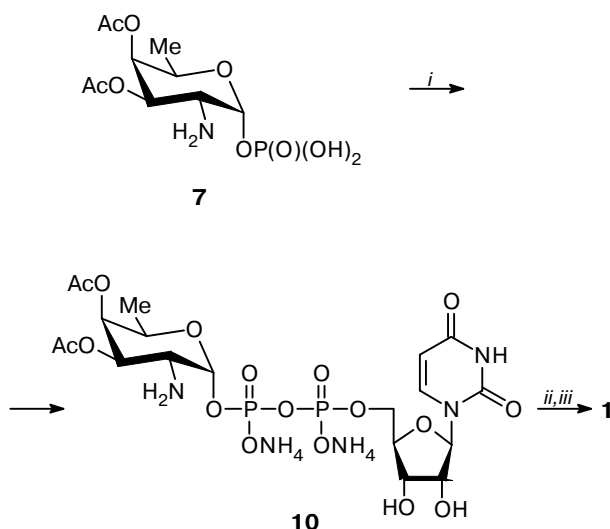
authors employed 2-chloropyridine as the base), anomerization of benzoylated hexose derivatives did not take place. Our results attest to easier anomerization of 6-deoxyhexose  $\beta$ -phosphoric triesters; this is in good agreement with the known data<sup>16</sup> on L-fucose derivatives.

The transition from  $\alpha$ -glycosyl dibenzyl phosphate **6** to *N*-acetyl- $\alpha$ -D-fucosamine 1-phosphate **9** was performed similarly to transformations described for the synthesis of *N*-acetyl- $\beta$ -L-fucosamine 1-phosphate.<sup>5</sup> Hydrogenolysis of the benzyl groups in compound **6** over Pd(OH)<sub>2</sub>/C accompanied by simultaneous reduction of the azido group gave 2-amino-3,4-di-*O*-acetyl-2-deoxy- $\alpha$ -D-fucopyranosyl phosphate (**7**) isolated as the internal salt in 87% yield. The chemical shifts and the multiplicities of the H(1) ( $\delta$  5.51, dd,  $J_{1,2} = 3.4$ ,  $J_{1,P} = 6.6$  Hz), H(2) ( $\delta$  3.62, br.dd,  $J_{2,3} = 10.8$  Hz), C(1) ( $\delta$  93.3, d,  $J_{C,P} = 5.1$  Hz), and C(2) ( $\delta$  49.8, d,  $J_{C,P} = 9.2$  Hz) signals are most significant for the proof of the product structure.

The transformation of amino sugar **7** into *N*-acetyl derivative **9**, i.e., *N*-acetylation and *O*-deacetylation, were performed by the reaction with *N*-acetoxy-succinimide (NAS)<sup>17</sup> in aqueous THF at pH 7.5 (2 h, 20 °C) followed by treatment of product **8** with dilute NaOH (1 h, 20 °C) for *O*-deacetylation. Neutralization and separation of the mixture by gel chromatography (TSK HW-40) resulted in the isolation of 2-acetamido-2-deoxy- $\alpha$ -D-fucopyranosyl phosphate **9** as the disodium salt in 86% yield. The presence of the signal for the *N*-acetyl group ( $\delta$  1.82) and the signal for H(1) corresponding to the  $\alpha$ -configuration of the anomeric center ( $\delta$  5.16, dd,  $J_{1,2} = 3.4$ ,  $J_{1,P} = 6.6$  Hz) in the <sup>1</sup>H NMR spectrum confirm the structure of compound **9**.

Phosphate **7** was converted into uridine 5'-(2-amino-3,4-di-*O*-acetyl-2-deoxy- $\alpha$ -D-fucopyranosyl) diphosphate (**10**) (Scheme 3) using the reaction of glycosyl phosphate with uridine 5'-phosphoimidazolide prepared *in situ* by treating uridine 5'-monophosphate with *N,N'*-carbonyldiimidazole. The sugar nucleotide was synthesized by the reaction of sugar phosphate **7** with the uridine 5'-phosphate activated derivative in DMF (1.1 : 1.0, 24 h, 20 °C). The product **10** was isolated by a procedure similar to a procedure described previously,<sup>5</sup> namely, using two-step gel chromatography, first, on a column with Sephadex G-25 (elution with a 25 mM solution of NH<sub>4</sub>OAc) and then on a column with TSK HW-40 (elution with water). The yield of compound **10** was 47%; its structure was confirmed by the data from the NMR spectra. The <sup>1</sup>H NMR spectrum exhibits characteristic signals for *O*-acetyl groups ( $\delta$  2.05, 2.20), for H(6'') ( $\delta$  1.20, d,  $J_{5,6} = 6.6$  Hz) and H(1'') ( $\delta$  5.50, dd,  $J_{1'',2''} = 3.4$ ,  $J_{1'',P} = 6.6$  Hz) atoms, for the  $\alpha$ -D-fucosamine residue, and signals for the uridine residue at  $\delta$  5.78 (m, H(5), H(1')) and 7.72 (H(6), d,  $J = 8.3$  Hz). The presence of two doublets with  $J = 19.3$  Hz at  $\delta$  -10.1 and -12.9 in the <sup>31</sup>P NMR spectrum confirms the structure of diphosphate **10**.

Scheme 3



**Reagents and conditions:** (i) uridine 5'-phosphoimidazolidine, DMF; (ii) NAS, THF—H<sub>2</sub>O (1 : 1); (iii) NaOH, H<sub>2</sub>O.

The transformation of product **10** into the target nucleoside diphosphate sugar **1** was achieved similarly to the transformation of amino phosphate **7** into *N*-acetate **9**. Product **1** was isolated as the disodium salt by gel chromatography on a column with TSK HW-40 (yield 93%). Its structure was confirmed by the data from <sup>1</sup>H, <sup>13</sup>C, and <sup>31</sup>P NMR spectroscopy. In particular, the presence of signals for the uridine residue at δ 7.86 (d, H(6), *J* = 8.3 Hz) and 5.81 (m, H(5), H(1')) as well as signals at δ 4.96 (dd, H(1''), *J*<sub>1'',2''</sub> = 3.4 Hz, *J*<sub>1'',P</sub> = 7.6 Hz); 2.05 (NHAc) and 1.20 (d, H(6''), *J*<sub>5'',6''</sub> = 6.6 Hz), corresponding to the 2-acetamido-2-deoxy-α-D-fucopyranose residue, in the <sup>1</sup>H NMR spectrum and two doublets in the <sup>31</sup>P NMR spectrum prove the structure of diphosphate **1**.

The new chemical synthesis of uridine 5'-(2-acetamido-2-deoxy-α-D-fucopyranosyl diphosphate) (**1**) and 2-acetamido-2-deoxy-α-D-fucopyranosyl phosphate (**9**) described here is promising for the introduction of a radioactive label into these compounds from [<sup>14</sup>C]-acetate and opens up broad possibilities for bio-synthetic studies.

### Experimental

<sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Bruker WM-250 spectrometer in CDCl<sub>3</sub> or in D<sub>2</sub>O at 300 K (acetone as the internal standard), <sup>31</sup>P NMR spectra were run on a Bruker AM-200 spectrometer in CDCl<sub>3</sub> or in D<sub>2</sub>O at 300 K (85% H<sub>3</sub>PO<sub>4</sub> as the external standard). UV spectra were measured on a Specord UV—VIS instrument. Thin layer chromatography was carried out on glass plates with a fixed silica gel layer (Merck); column chromatography was performed on silica gel LiChroprep Si 60 (Merck), and gel chromatography, on columns with Sephadex G-25 superfine (600×25 mm) and TSK

HW-40 (800×20 mm). The separation was monitored using a Knauer differential refractometer. The purification of solvents was described previously.<sup>5</sup> *N*-Acetoxysuccinimide was synthesized by a known procedure.<sup>17</sup> Cesium dibenzyl phosphate was prepared by mixing hydrogen dibenzyl phosphate with 1.01 equiv. of Cs<sub>2</sub>CO<sub>3</sub> in acetonitrile. The solvent was evaporated and the residue was dried *in vacuo* at 25 °C.

**3,4-Di-*O*-acetyl-1,5-anhydro-2,6-dideoxy-*D*-lyxo-hex-1-enitol (**2**)** was prepared as described previously<sup>5</sup> from 2,3,4-tri-*O*-acetyl-α-D-fucopyranosyl bromide (5.2 g, 14.7 mmol).<sup>8</sup> The yield of compound **2** was 2.21 g (70%); it was used subsequently without additional purification. An analytical sample of compound **2** was isolated by column chromatography in the light petroleum—EtOAc (6 : 1) system, m.p. 49 °C, [α]<sub>D</sub><sup>22</sup> −8.77 (*c* 1.8, acetone) (*cf.* lit. data for the *L*-isomer<sup>18</sup>). Found (%): C, 56.39; H, 6.65. C<sub>10</sub>H<sub>14</sub>O<sub>5</sub>. Calculated (%): C, 56.10; H, 6.59. <sup>1</sup>H NMR (CDCl<sub>3</sub>), δ: 1.22 (d, 3 H, H(6), *J*<sub>5,6</sub> = 6.7 Hz); 1.96, 2.10 (both s, 3 H each, OAc); 4.20 (br.q, 1 H, H(5)); 4.62 (dt, 1 H, H(3), *J*<sub>2,3</sub> = *J*<sub>1,3</sub> = 6.0 Hz, *J*<sub>3,4</sub> = 1.2 Hz); 5.24 (br.d, 1 H, H(2), *J*<sub>1,2</sub> = 1.0 Hz); 5.56 (br.d, 1 H, H(4)); 6.45 (dd, 1 H, H(1)). <sup>13</sup>C NMR (CDCl<sub>3</sub>), δ: 16.6 (C(6)); 20.8, 20.9 (both CH<sub>3</sub>CO); 65.1 (C(4)); 66.4 (C(3)); 71.6 (C(5)); 98.3 (C(2)); 146.1 (C(1)); 170.4, 170.7 (both CH<sub>3</sub>CO).

**2-Azido-3,4-di-*O*-acetyl-2-deoxy-α-D-fucopyranosyl nitrate (**3**)**. Sodium azide (1.75 g, 27.2 mmol) and Ce(NH<sub>4</sub>)<sub>2</sub>(NO<sub>3</sub>)<sub>6</sub> (29.6 g, 54.4 mmol) were added with stirring at −25 °C to a solution of compound **2** (2.9 g, 13.7 mmol) in 70 mL of MeCN. The mixture was stirred for 4 h at −25 °C and diluted with 50 mL of Et<sub>2</sub>O; the solid precipitate was filtered off and washed with 30 mL of Et<sub>2</sub>O. The organic layer was washed with water (3×20 mL) and dried with Na<sub>2</sub>SO<sub>4</sub>. The solvent was evaporated *in vacuo* and the residue was recrystallized from Et<sub>2</sub>O to give 2.75 g (65%) of compound **3**, m.p. 118–119 °C, [α]<sub>D</sub><sup>28</sup> +130 (*c* 1.0, CHCl<sub>3</sub>). Found (%): C, 37.58; H, 4.44; N, 17.69. C<sub>10</sub>H<sub>14</sub>N<sub>4</sub>O<sub>8</sub>. Calculated (%): C, 37.74; H, 4.43; N, 17.61. IR (KBr), ν/cm<sup>−1</sup>: 2115 (N<sub>3</sub>); 1665 (ONO<sub>2</sub>). <sup>1</sup>H NMR, δ: 1.20 (d, 3 H, H(6), *J*<sub>5,6</sub> = 6.1 Hz); 2.09, 2.21 (both s, 3 H each, OAc); 4.10 (dd, 1 H, H(2), *J*<sub>1,2</sub> = 4.2 Hz, *J*<sub>2,3</sub> = 11.1 Hz); 4.31 (br.q, 1 H, H(5)); 5.26 (dd, 1 H, H(3), *J*<sub>3,4</sub> = 3.5 Hz); 5.36 (br.d, 1 H, H(4)); 6.32 (d, 1 H, H(1)). <sup>13</sup>C NMR, δ: 16.0 (C(6)); 20.5, 20.6 (both COCH<sub>3</sub>); 56.1 (C(2)); 68.2 (C(4)); 69.1 (C(3)); 69.8 (C(5)); 97.5 (C(1)); 169.6, 170.2 (both COCH<sub>3</sub>).

**(2-Azido-3,4-di-*O*-acetyl-2-deoxy-α-D-fucopyranosyl) diphenyl phosphate (**4**)**. Cesium diphenyl phosphate (616 mg, 1.62 mmol) and 18-crown-6 (424 mg, 1.62 mmol) were added with stirring at 20 °C to a solution of compound **3** (340 mg, 1.08 mmol) in 0.5 mL of anhydrous DMF. The reaction mixture was stirred for 5 h, poured into 15 mL of brine, and extracted with CHCl<sub>3</sub> (2×3 mL). The organic phase was washed with water, dried with Na<sub>2</sub>SO<sub>4</sub>, and concentrated *in vacuo*. The residue was chromatographed in the light petroleum—EtOAc system (4 : 1) to give 424 mg (78%) of compound **4**, *R*<sub>f</sub> 0.36 (light petroleum—EtOAc, 2 : 1), m.p. 92–93 °C, [α]<sub>D</sub><sup>24</sup> +71.3 (*c* 1.0, CHCl<sub>3</sub>). Found (%): C, 49.82; H, 5.10; N, 8.81. C<sub>20</sub>H<sub>24</sub>N<sub>3</sub>O<sub>9</sub>P. Calculated (%): C, 49.90; H, 5.03; N, 8.73. <sup>1</sup>H NMR (C<sub>6</sub>D<sub>6</sub>), δ: 0.78 (d, 3 H, H(6), *J*<sub>5,6</sub> = 6.6 Hz); 1.67, 1.73 (both s, 3 H each, OAc); 3.62 (ddd, 1 H, H(2), *J*<sub>1,2</sub> = 3.4 Hz, *J*<sub>2,3</sub> = 11.1 Hz, *J*<sub>1,P</sub> = 3.1 Hz); 3.81 (br.q, 1 H, H(5)); 5.28 (br.d, 1 H, H(4)); 5.52 (dd, 1 H, H(3), *J*<sub>3,4</sub> = 4.1 Hz); 6.08 (dd, 1 H, H(1), *J*<sub>1,P</sub> = 6.4 Hz); 6.81–7.51 (m, 10 H, Ph). <sup>13</sup>C NMR (CDCl<sub>3</sub>), δ: 15.6 (C(6)); 20.5, 20.6 (both CH<sub>3</sub>CO); 57.4 (d, C(2), *J*<sub>C,P</sub> = 8.7 Hz); 67.3 (C(3)); 68.9 (C(4)); 69.1 (C(5)); 97.2 (d, C(1), *J*<sub>C,P</sub> = 5.5 Hz); 120.0, 120.1, 120.3, 125.6, 129.7 (all Ar); 169.7, 170.2 (both COCH<sub>3</sub>). <sup>31</sup>P NMR (CDCl<sub>3</sub>): δ −12.7.



**(2-Azido-3,4-di-O-acetyl-2-deoxy- $\beta$ -D-fucopyranosyl) dibenzyl phosphate (5).** Cesium dibenzyl phosphate (670 mg, 1.57 mmol) was added with stirring at 20 °C to a solution of compound **3** (350 mg, 1.10 mmol) in 0.7 mL of anhydrous DMF. The reaction mixture was stirred for 2 h, poured into 5 mL of brine, and extracted with  $\text{CHCl}_3$  ( $3 \times 5$  mL). The organic phase was washed with water, dried with  $\text{Na}_2\text{SO}_4$ , and concentrated *in vacuo*, and the residue was chromatographed in the light petroleum–EtOAc system (4 : 1) to give 460 mg (79%) of compound **5**, syrup,  $R_f$  0.27 (light petroleum–EtOAc, 2 : 1).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ),  $\delta$ : 1.18 (d, 3 H, H(6),  $J_{5,6}$  = 6.9); 2.03, 2.15 (both s, 3 H each, 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),  $\text{PhCH}_2$ ); 5.18 (br.d, 1 H, H(4)); 7.31 (m, 10 H, Ph).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ),  $\delta$ : 15.6 (C(6)); 20.5 ( $\text{CH}_3\text{CO}$ ); 60.8 (d, C(2),  $J_{\text{C,P}}$  = 9.6 Hz); 68.7 (C(4)); 69.0 (d,  $\text{PhCH}_2$ ,  $J_{\text{C,P}}$  = 4.1 Hz); 69.1 (d,  $\text{PhCH}_2$ ,  $J_{\text{C,P}}$  = 4.3 Hz); 70.1 (C(3)); 71.4 (C(5)); 97.5 (d, C(1),  $J_{\text{C,P}}$  = 5.5 Hz); 127.7, 127.8, 128.5, 135.2, 135.3, 135.5 (Ar); 169.6, 170.2 ( $\text{COCH}_3$ ).  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  –2.75.

**(2-Azido-3,4-di-O-acetyl-2-deoxy- $\alpha$ -D-fucopyranosyl) dibenzyl phosphate (6).**  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  (32  $\mu\text{L}$ , 0.25 mmol) was added with stirring at 20 °C to a solution of compound **5** (248 mg, 0.49 mmol) and 2-bromopyridine (151 mg, 0.98 mmol) in 2 mL of anhydrous  $\text{CH}_2\text{Cl}_2$ . The mixture was stirred for 1 h, the solvent was evaporated *in vacuo*, and the residue was chromatographed in the light petroleum–EtOAc system (5 : 1) to give 178 mg (72%) of compound **6**,  $R_f$  0.36 (heptane–EtOAc, 1 : 1), m.p. 94–95 °C,  $[\alpha]_{\text{D}}^{25}$  +77.5 ( $c$  2.35,  $\text{CHCl}_3$ ). Found (%): C, 52.01; H, 5.49; N, 8.26.  $\text{C}_{22}\text{H}_{28}\text{N}_3\text{O}_9\text{P}$ . Calculated (%): C, 51.87; H, 5.54; N, 8.23.  $^1\text{H}$  NMR ( $\text{C}_6\text{D}_6$ ),  $\delta$ : 0.77 (d, 3 H, H(6),  $J_{5,6}$  = 6.8 Hz); 1.65, 1.73 (both s, 3 H each, OAc); 3.60 (ddd, 1 H, H(2),  $J_{1,2}$  = 3.4 Hz,  $J_{2,3}$  = 11.1 Hz,  $J_{1,\text{P}}$  = 3.1 Hz); 3.78 (br.q, 1 H, H(5)); 4.95, 5.12 (both d, 2 H each,  $\text{CH}_2\text{Ph}$ ,  $J_{\text{H,P}}$  = 8.1 Hz); 5.22 (br.d, 1 H, H(4)); 5.45 (dd, 1 H, H(3),  $J_{3,4}$  = 4.1 Hz); 5.95 (dd, 1 H, H(1),  $J_{1,\text{P}}$  = 6.3 Hz); 7.00–7.35 (m, 10 H, Ph).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ),  $\delta$ : 15.6 (C(6)); 20.5, 20.6 (both  $\text{CH}_3\text{CO}$ ); 57.3 (d, C(2),  $J_{\text{C,P}}$  = 8.6 Hz); 66.8 (C(3)); 68.7 (C(4)); 69.4 (C(5)); 69.5, 69.6 (both d,  $\text{PhCH}_2$ ,  $J_{\text{H,P}}$  = 5.6 Hz); 96.1 (d, C(1),  $J_{\text{C,P}}$  = 5.6 Hz); 127.8, 127.9, 128.6, 135.4, 135.5 (Ar); 169.7, 170.2 (both  $\text{COCH}_3$ ).  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  –2.05.

**2-Amino-3,4-di-O-acetyl-2-deoxy- $\alpha$ -D-fucopyranosyl phosphate (7).** Compound **6** (178 mg, 0.354 mmol) was dissolved in 3 mL of anhydrous dioxane and hydrogenated at 25 °C under atmospheric pressure over 10%  $\text{Pd}(\text{OH})_2/\text{C}$  (40 mg) for 4 h. The catalyst was filtered off and washed with dioxane (1  $\times$  5 mL) and EtOH (2  $\times$  5 mL). Lyophilization of the solution gave 91 mg (87%) of compound **7**,  $R_f$  0.32 (propan-2-ol–1  $M$  aqueous  $\text{NH}_4\text{OAc}$ , 2 : 1).  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ ),  $\delta$ : 0.94 (d, 3 H, H(6),  $J_{5,6}$  = 6.6 Hz); 1.82, 1.96 (both s, 3 H each, OAc); 3.62 (br.dd, 1 H, H(2),  $J_{1,2}$  = 3.4 Hz,  $J_{2,3}$  = 10.8 Hz); 4.31 (br.q, 1 H, H(5)); 5.10–5.21 (m, 2 H, H(3), H(4)); 5.51 (dd, 1 H, H(1),  $J_{1,\text{P}}$  = 6.6 Hz).  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ ),  $\delta$ : 16.2 (C(6)); 21.2, 21.3 (both  $\text{CH}_3\text{CO}$ ); 49.8 (d, C(2),  $J_{\text{C,P}}$  = 9.2 Hz); 67.5 (C(3)); 69.5 (C(4)); 71.2 (C(5)); 93.3 (d, C(1),  $J_{\text{C,P}}$  = 5.1 Hz); 173.9, 174.9 (both  $\text{COCH}_3$ ).  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  –0.56.

**Disodium 2-acetamido-2-deoxy- $\alpha$ -D-fucopyranosyl phosphate (9).** A solution of *N*-acetoxy succinimide (NAS)<sup>17</sup> (44 mg, 0.29 mmol) in 0.44 mL of aqueous THF (1 : 1) was added with stirring at 20 °C to a solution of compound **7** (42 mg, 0.14 mmol) in 0.22 mL of water. The reaction mixture was stirred for 2 h, pH 7.5 being maintained by addition of 0.1  $M$  NaOH, and cooled to 0 °C. A 1  $M$  solution of NaOH (0.1 mL) was added, and the mixture was kept for 1 h at 20 °C. The reaction mixture

was neutralized with Dowex-50 ( $\text{H}^+$ ) cation exchange resin to pH 7.5 according to pH-meter reading. The resin was filtered off and washed with water ( $3 \times 2$  mL). The filtrate was concentrated *in vacuo* to 0.1 mL and applied onto a column with TSK HW-40, and the column was washed with water. The fractions containing the product were combined, concentrated *in vacuo* to 5 mL, and treated with Dowex-50 ( $\text{Na}^+$ ) cation exchange resin. The resin was filtered off and washed with water ( $3 \times 5$  mL). Lyophilization from water gave 36 mg (86%) of compound **7**,  $[\alpha]_{\text{D}}^{27}$  +72.1 ( $c$  1.5,  $\text{H}_2\text{O}$ ).  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ ),  $\delta$ : 1.10 (d, 3 H, H(6),  $J_{5,6}$  = 6.6 Hz); 1.82 (s, 3 H, NHAc); 3.52–3.97 (m, 3 H, H(2), H(3), H(4)); 4.05 (br.q, 1 H, H(5)); 5.16 (dd, 1 H, H(1),  $J_{1,2}$  = 3.4 Hz,  $J_{1,\text{P}}$  = 6.6 Hz).

**Diammonium uridine 5'-(2-amino-3,4-di-O-acetyl-2-deoxy- $\alpha$ -D-fucopyranosyl diphosphate) (10).** 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 and the mixture was stirred for 15 min. The reaction mixture was lyophilized and the residue was dissolved in 0.5 mL of anhydrous DMF. *N,N'*-Carbonyldiimidazole (150 mg, 0.92 mmol) was added to the resulting solution, the mixture was stirred under Ar for 3 h at 20 °C, and anhydrous MeOH (0.035 mL) was added. The mixture was stirred for an additional 20 min and evacuated (1 Torr, 20 °C) for 15 min to remove excess MeOH. A solution of compound **7** (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 reaction mixture was stirred for 24 h under Ar, diluted with a 25  $mM$  aqueous  $\text{NH}_4\text{OAc}$  (3 mL), and washed with  $\text{CHCl}_3$  (1  $\times$  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  $\text{NH}_4\text{OAc}$ , the separation being monitored using an UV detector (254 nm). The fractions containing the product were concentrated *in vacuo* to 1.5 mL, and the solution was applied onto a column with TSK HW-40, and the column was washed with water. Lyophilization of fractions containing the product gave 91 mg (47%) of compound **10**,  $R_f$  0.66 (propan-2-ol–1  $M$  aqueous solution of  $\text{NH}_4\text{OAc}$ , 2 : 1).  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ ),  $\delta$ : 1.20 (d, 3 H, H(6''),  $J_{5'',6''}$  = 6.6 Hz); 2.05, 2.20 (both s, 3 H each, OAc); 4.07–4.36 (m, 7 H, H(2''), H(2'), H(3'), H(4'), H(5'a), H(5'b), H(5'')); 5.08 (dd, 1 H, H(3''),  $J_{2'',3''}$  = 11.1 Hz,  $J_{3'',4''}$  = 3.4 Hz); 5.23 (br.d, 1 H, H(4'')); 5.50 (dd, 1 H, H(1''),  $J_{1'',2''}$  = 3.4 Hz,  $J_{1'',\text{P}}$  = 6.6 Hz); 5.78 (m, 2 H, H(5), H(1')); 7.73 (d, 1 H, H(6),  $J_{5,6}$  = 8.3 Hz).  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ ),  $\delta$ : 16.2 (C(6'')); 21.1, 21.3 (both  $\text{COCH}_3$ ); 49.7 (d, C(2''),  $J_{\text{C,P}}$  = 9.3 Hz); 65.3 (d, C(5'),  $J_{\text{C,P}}$  = 5.4 Hz); 67.8 (C(3'')); 69.5 (C(4'')); 70.7 (C(5'')); 71.1 (C(2'')); 75.1 (C(3')); 84.1 (d, C(4'),  $J_{\text{C,P}}$  = 8.8 Hz); 89.7 (C(1')); 94.0 (d, C(1''),  $J_{\text{C,P}}$  = 5.4 Hz); 103.7 (C(5)); 142.9 (C(6)); 152.9 (C(2)); 167.3 (C(4)); 173.8, 174.8 (both  $\text{COCH}_3$ ).  $^{31}\text{P}$  NMR ( $\text{D}_2\text{O}$ ),  $\delta$ : –10.1, –12.9 (both d,  $J$  = 19.3 Hz).

**Disodium uridine 5'-(2-acetamido-2-deoxy- $\alpha$ -D-fucopyranosyl diphosphate) (1).** A solution of NAS (22.4 mg, 0.12 mmol) in 0.4 mL of aqueous THF (1 : 1) was added with stirring at 20 °C to a solution of compound **10** (24 mg, 0.038 mmol) in 0.3 mL of water. The reaction mixture was stirred for 5 h, pH 7.5 being maintained by addition of 0.1  $M$  NaOH, and cooled to 0 °C. A 1  $M$  solution of NaOH (0.2 mL) was added, and the mixture was kept for 3 h at 20 °C and neutralized with Dowex-50 ( $\text{H}^+$ ) cation exchange resin to pH 7.5 according to pH-meter reading. The cation exchange resin was filtered off and washed with water ( $3 \times 3$  mL), and the filtrate was concentrated *in vacuo* to 0.2 mL and subjected to gel chromatography on a column with TSK HW-40; water was used for elution. The fractions containing the product were

lyophilized, yield 20 mg (93%). The content of compound **1** (based on absorption at 262 nm) was 96%. UV:  $\lambda_{\text{max}}$  262 nm.  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ ),  $\delta$ : 1.20 (d, 3 H, H(6''),  $J_{5'',6''} = 6.6$  Hz); 2.05 (s, 3 H, NHAc); 3.82 (br.d, 1 H, H(4''),  $J_{3'',4''} = 2.4$  Hz); 3.94 (dd, 1 H, H(3''),  $J_{2'',3''} = 10.6$  Hz); 4.10–4.41 (m, 7 H, H(2'), H(3'), H(4'), H(5'a), H(5'b), H(2''), H(5'')); 4.96 (dd, 1 H, H(1''),  $J_{1'',2''} = 3.4$  Hz,  $J_{1'',\text{p}} = 6.6$  Hz); 5.81 (m, 2 H, H(5), H(1')); 7.86 (d, 1 H, H(6),  $J_{5,6} = 8.3$  Hz).  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ ),  $\delta$ : 16.1 (C(6'')); 22.9 ( $\text{COCH}_3$ ); 50.2 (d, C(2''),  $J_{\text{C,p}} = 8.6$  Hz); 65.6 (d, C(5'),  $J_{\text{C,p}} = 5.4$  Hz); 68.6, 68.8, 71.2, 71.8 (C(3''), C(4''), C(5''), C(2')); 75.1 (C(3')); 83.8 (d, C(4'),  $J_{\text{C,p}} = 8.8$  Hz); 89.1 (C(1')); 95.2 (d, C(1''),  $J_{\text{C,p}} = 5.4$  Hz); 102.6 (C(5)); 141.7 (C(6)); 151.6 (C(2)); 166.1 (C(4)); 174.1 ( $\text{COCH}_3$ ).  $^{31}\text{P}$  NMR ( $\text{D}_2\text{O}$ ),  $\delta$ : -10.1, -12.5 (both d,  $J = 19.3$  Hz).

This work was financially supported by the International Wellcome Trust Foundation (Grant 049419/Z/96/Z), the Russian Foundation for Basic Research (Project No. 96-03-32473), and the Foundation for the Support of Leading Scientific Schools of the Russian Federation (Grant No. 96-15-97380).

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Received February 15, 2001