# Glycosylation of dibutyl phosphate anion with arabinofuranosyl bromide: unusual influence of concentration of the reagents on the ratio of anomeric glycosyl phosphates formed*

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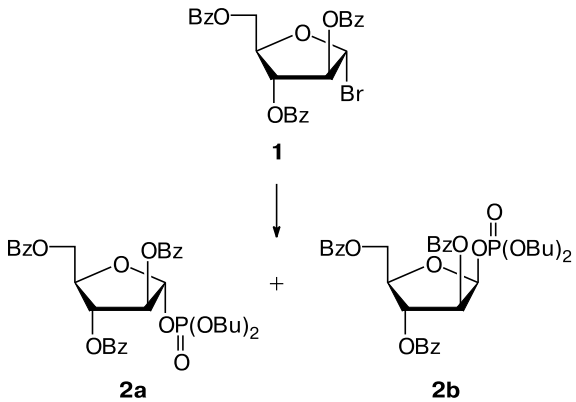
Glycosyl phosphates are widely used building blocks in the synthesis of various natural products, for example, nucleoside diphosphates,[1—3] proteophosphoglycans,[4] and C- and O-alkyl(aryl) glycosides[5,6]. Strategies of oligosaccharide synthesis using glycosyl phosphates as glycosyl donors[7,8] were developed for various sugars (for example, derivatives of mannose,[9] sialic acids[10] and arabinofuranose[11]).

Several known methods for the synthesis of the glycosyl phosphates are based on glysosylation reaction as the key step.[3,12,13] During the synthesis (Scheme 1) of dibutyl (2,3,5-tri-*O*-benzoyl-D-arabinofuranosyl) phosphate (**2**) by glycosylation of dibutyl phosphoric acid ($(BuO)_2P(O)OH$) with 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl bromide (**1**)[14] in MeCN in the presence of $Pr^i_2NEt$[15] we found that concentration of the reagents influences the anomeric ratio of glycosyl phosphates **2**. A decrease of concentration (*c*) of glycosyl bromide **1** (while keeping the molar ratio between all reagents constant) leads to an increase in the reaction stereoselectivity in favor of the α-anomer **2a**.

**Scheme 1**




**Reagents and conditions:** $(BuO)_2P(O)OH$, $Pr^i_2NEt$, MeCN, 2 h, 20 °C.

A more detailed study of the concentration dependence of the stereoselectivity in a wide range of concentrations of glycosyl bromide **1** (0.001—0.2 mol $L^{-1}$) revealed that the anomeric ratio of glycosyl phosphates **2** depends on concentration of the reaction mixture in a complex manner (Table 1, Fig. 1). While in the most concentrated solution ($c$ = 0.2 mol $L^{-1}$) the glycosylation proceeds unselectively (α/β = 1.5 : 1), upon transition to more diluted solutions the share of α-anomer **2a** increases, reaching a high value (α/β = 20 : 1) at $c$ = 0.01 mol $L^{-1}$. Upon a further decrease of concentration of glycosyl bromide **1** the stereoselectivity increases sharply (Table 1, Fig. 1) and the reaction becomes virtually stereospecific (α/β > 50 : 1).

A considerable influence of concentration of reagents on the outcome of glycosylation has been reported in several cases,[16—20] both the product yield[16c,e,f,17,18] and stereoselectivity[16e,f,17—20] as well as the reactivity[16e,f,17] of the glycosyl donor being affected by dilution of the reac-

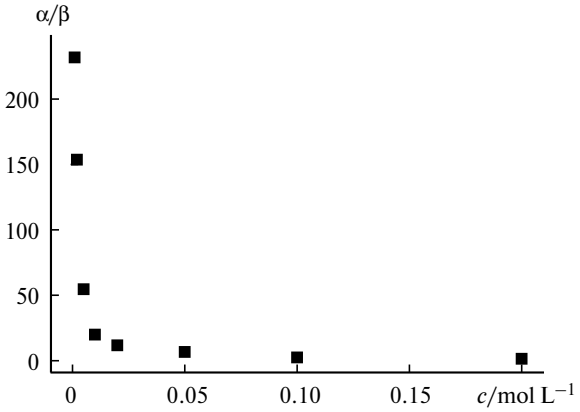



**Fig. 1.** Dependence of the anomeric ratio (α/β) of glycosyl phosphates **2** on the concentration ($c$) of glycosyl bromide **1**.

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**Table 1.** Influence of concentration of glycosyl bromide **1** on the outcome of glycosylation

| [**1**]/(mol $L^{-1}$)[a] | **2a**/**2b** (α/β)[b] | Yield **2** (%) |
|---|---|---|
| 0.001 | 232[c] | 76 |
| 0.002 | 154[c] | 75 |
| 0.005 | 55[c] | 78 |
| 0.01 | 20 | 75 |
| 0.02 | 11.8 | 75 |
| 0.05 | 6.7 | 77 |
| 0.1 | 2.5 | 82 |
| 0.2 | 1.5 | 85 |

[a] In all experiments the same amount of glycosyl bromide **1** (50 mg, 95 μmol) was used. Concentration of the other reagents was proportional to the concentration of **1** (molar ratio **1** : $Pr^i_2NEt$ : $(BuO)_2P(O)OH$ = 1 : 4 : 4). [b] Anomeric ratio of the glycosyl phosphate **2** (α/β) was calculated by integration of the signals of α- and β-anomers of glycosyl phosphates **2** in $^{31}P$ NMR spectra of the reaction mixtures after work up. [c] In the low concentration range (0.001—0.005 mol $L^{-1}$), integration of the signal of β-anomer **2b** was not precise due to a low signal-to-noise ratio. At the same time, in the $^{31}P$ NMR spectra, the intensity of the signal of β-anomer **2b** significantly decreases upon dilution of the reaction mixture.

tion mixture. In most publications, results of the glycosylation at only two concentrations, "dilute" (0.001—0.005 mol $L^{-1}$) and "regular" (0.05 mol $L^{-1}$), were usually compared. A detailed analysis of the influence of concentration on the glycosylation outcome was performed only in our earlier studies[16c,e,f] on sialylation.

The reasons of the observed unusual influence of concentration on the stereoselectivity of glycosylation with ararbinofuranosyl bromide **1** can be related to a change in the glycosylation mechanism[15,21] or a change in the structure of the reaction solution upon dilution[16]. Clarification of this issue needs further research, which is the subject of our current studies.

To a solution of 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl bromide (**1**) (50 mg, 0.095 mmol) in anhydrous MeCN (half of the volume, required to obtain the desired concentration (see Table 1)), a solution prepared from $(BuO)_2P(O)OH$ (0.38 mmol, 77 μL) and $Pr^i_2NEt$ (0.38 mmol, 66 μL) in the second half of the required volume of anhydrous MeCN was added. The reaction mixture was stirred at ~20 °C for 2 h. Then saturated aqueous $NaHCO_3$ (15 mL) was added and the resulting mixture was extracted with toluene (3×20 mL). The combined organic layer was washed with saturated aqueous $NaHCO_3$ (20 mL) and filtered through a layer of $Na_2SO_4$ (~10 mm). The filtrate was concentrated *in vacuo* on a water-aspirator pump, the residue was dried *in vacuo* on an oil pump. The obtained mixture of the α- and β-glycosyl phosphates **2a** and **2b** was analyzed by NMR and TLC on silica gel; yields and anomeric ratios are listed in Table 1.

**Dibutyl (2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl) phosphate (2a)**, $R_f$ 0.32 (EtOAc—toluene, 1 : 5.7 (v/v)), $[\alpha]_D^{21}$ −14.4 (*c* 1.0, $CHCl_3$). Mass-spectrum: *m/z* 677.2118 $[M + Na]^+$. Calculated for $C_{34}H_{39}NaO_{11}P^+$: 677.2122. $^1H$ NMR (300 MHz, $CDCl_3$), δ: 0.84—0.98 (m, 6 H, $Bu^n$); 1.32—1.49 (m, 4 H, $Bu^n$); 1.55—1.76 (m, 4 H, $Bu^n$); 4.15 (qd, 4 H, $Bu^nO$, *J* = 6.7 Hz, *J* = 3.8 Hz); 4.72 (dd, 1 H, H(5a), *J* = 13.0 Hz, *J* = 6.5 Hz); 4.78—4.86 (m, 2 H, H(4), H(5b)); 5.61 (d, 2 H, H(3), *J* = 3.9 Hz); 5.69 (s, 2 H, H(2)); 6.09 (d, 1 H, H(1), *J* = 5.0 Hz); 7.25—7.34 (m, 2 H, Ph); 7.37—7.55 (m, 5 H, Ph); 7.56—7.65 (m, 2 H, Ph); 7.98—8.06 (m, 4 H, Ph); 8.06—8.13 (m, 1 H, Ph). $^{13}C$ NMR (75 MHz, $CDCl_3$), δ: 13.5, 18.6, 32.1, 32.2 ($Bu^n$); 63.4 (C(5)); 67.8 (d, $Bu^nO$ (1), *J* = 5.5 Hz); 67.9 (d, $Bu^nO$ (2), *J* = 5.5 Hz); 77.2 (C(3)); 82.0 (d, C(2), *J* = 11.1 Hz); 83.2 (C(4)); 102.8 (d, C(1), *J* = 5.3 Hz); 128.3, 128.5, 128.5, 129.7, 129.9, 133.1, 133.6 (Ph); 165.0, 165.6, 166.0 (CO). $^{31}P$ NMR (121 MHz, $CDCl_3$), δ: −3.2.

**Dibutyl (2,3,5-tri-*O*-benzoyl-β-D-arabinofuranosyl) phosphate (2b)**, $R_f$ 0.21 (EtOAc—toluene, 1 : 5.7 (v/v)). $^1H$ NMR (300 MHz, $CDCl_3$), δ (selected signals): 5.99—6.06 (m, 1 H, H(2)); 6.26 (dd~t, 1 H, H(1), *J* = 5.0 Hz). $^{13}C$ NMR (75 MHz, $CDCl_3$), δ: 65.3 (C(5)); 75.0 (C(3)); 79.7 (C(4)); 97.7 (d, C(1), *J* = 4.8 Hz). $^{31}P$ NMR (121 MHz, $CDCl_3$), δ: −2.4.

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