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A Short Synthesis of 4-Deoxy-4-Fluoroglucosaminides: Methylumbelliferyl *N*-Acetyl-4-Deoxy-4-Fluoro-β-*D*-Glucosaminide

N. A. Markina[a] and Ya. V. Voznyi[b,1]

[a] *Higher Chemical College, Russian Academy of Sciences, Moscow, Russia*
[b] *Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninskii pr. 47, Moscow, 119991 Russia*

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Abstract—A convenient method of synthesis of 1,6-anhydro-4-deoxy-2-*O*-tosyl-4-fluoro-β-*D*-glucopyranose by fusion of 1,6;3,4-dianhydro-2-*O*-tosyl-β-*D*-galactopyranose with 2,4,6-trimethylpyridinium fluoride was found. By a successive action of ammonia, methyl trifluoroacetate, and acetic anhydride, the resulting compound was transformed into 1,6-anhydro-3-*O*-acetyl-2,4-dideoxy-2-trifluoroacetamido-4-fluoro-β-*D*-glucopyranose, which was converted into 3,6-di-*O*-acetyl-2,4-dideoxy-2-trifluoroacetamido-4-fluoro-α*D*-glucopyranosyl fluoride by the reaction with HF/Py. The resulting fluoride was further used as a glycosyl donor in the synthesis of methylumbelliferyl *N*-acetyl-4-deoxy-4-fluoro-β-*D*-glucosaminide.

Key words: an ionic liquid, 1,6-anhydro-4-deoxy-4-fluoro-β-D-glucopyranose, levoglucosan, methylumbelliferyl N-acetyl-4-deoxy-4-fluoro-β-D-glucosaminide, 2,4,6-trimethylpyridinium fluoride

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INTRODUCTION

The replacement of hydroxy groups by fluorine atom in carbohydrate molecules leads to analogues of great value for studying biochemical transformations of sugars [1].[2] Therefore, an interest in the development of new and improvement of known methods of synthesis of deoxyfluorosugars is not reduced. Reviews and the whole issues of scientific journals devoted to this problem [1–3] have recently appeared. Modern methods of preparation of such compounds are based on the application of DAST [4–8], which replaces hydroxy group by fluorine atom with the reversal of configuration. A wide application also finds another way consisting in mesylation of free hydroxy group by methanesulfonyl chloride with the subsequent replacement of mesylate by fluorine by the action of tetrabutylammonium fluoride [8–10]. A drawback of these approaches is a great volume of preliminary work connected with the protection of hydroxy groups not participating in the reaction and frequently arising necessity of the double reversal of configuration at certain carbon atoms.

Our goal was the development of a simple and short way to glycosyl donors on a the basis of 4-deoxy-4-fluoroglucosamine and synthesis of methylumbelliferyl 4-fluoro-4-deoxy-*N*-acetyl-β-*D*-glucosaminide (**VII**), a fluorogenic substrate necessary for studying the substrate specificity of enzymes, splitting the glycoside linkage in *N*-acetylglucosaminides. The corresponding protected glycosyl fluoride was chosen as the most prospective glycosyl donor, as it is known that glycosyl fluorides are more stable than other glycosyl halides and, if necessary, can be easily transformed into other types of glycosyl donors, e.g., in thioglycosides.

RESULTS AND DISCUSSION

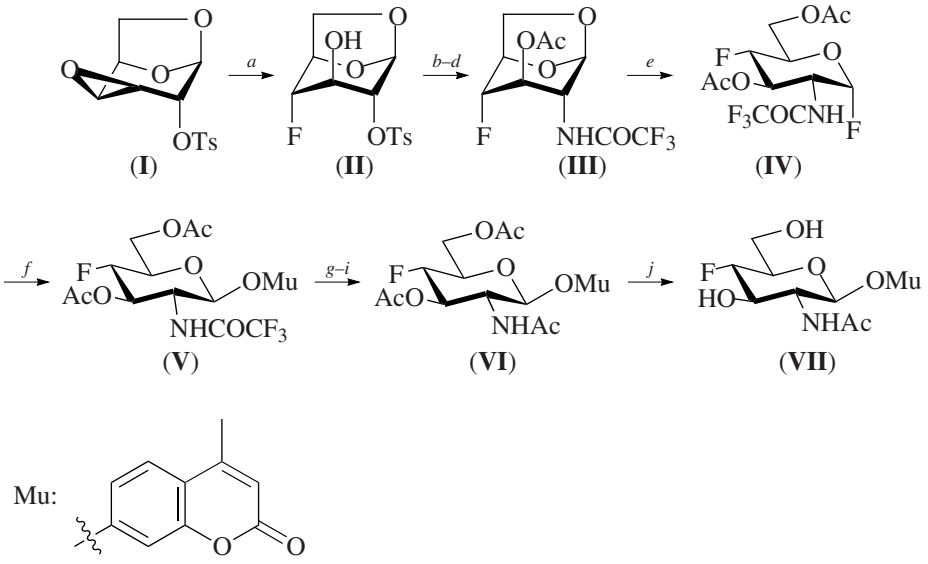
The reactions of 3,4-oxyrane cycle opening by fluoride ions in 1,6;3,4-dianhydro-2-*O*-tosyl-β-*D*-galactopyranose (**I**) are known to hardly proceed [11]. For example, the interaction of dianhydride (**I**) with KHF_2 in boiling ethyleneglycol [12] carried out with the purpose of synthesis of 1,6-anhydro-4-deoxy-2-*O*-tosyl-4-fluoro-β-*D*-glucopyranose (**II**) did not lead to the desirable result: only 1,6-anhydro-2,4-dideoxy-2,4-difluoro-β-*D*-glucopyranose was isolated from the reaction mixture in 5% yield. Synthesis of compound (**II**) from (**I**) was carried out in 2% yield by the action of 25% HF in dioxane [13]. Compound (**II**) has later been obtained by tosylation of hydroxy group at C2 in 1,6-anhydro-4-deoxy-4-fluoro–β-*D*-glucopyranose and its structure was proved by conversion into the known derivatives of 4-deoxy-4-fluoroglucopyranose [14].

The opening of oxyrane cycle is formally the addition of HF, and this reaction known to be greatly

[1] Corresponding author; phone: +7 (495) 137-6791; e-mail: voznyi@ioc.ac.ru

[2] Abbreviations: DAST, diethylaminosulfotrifluoride; DMAP, *N,N*-dimethylaminopyridine; Mu, 4-methylumbelliferyl; and Me_3SiOMu, 4-methyl-7-trimethylsilyloxycoumarin.

affected by the nature of the hydrogen fluoride donor (cf. the review [15]). To improve the yield of product (**II**) at the opening of oxyrane cycle in (**I**), we suggested the use of collidine hydrofluoride as a reaction medium and HF source. As a result of optimization of reaction conditions, it was established that carrying out of the reaction in melt at a temperature of 120–125°C led to isolation of (**II**) in preparative yield of 45–47% or in 75–85% yield if one takes into account the starting substance (**I**) not entered the reaction (scheme).



a: $Me_3Py \cdot HF$, 125°C, 55 h; *b*: NH_3/MeOH, 100°C, 16 h; *c*: MeO_2CCF_3/MeOH/Et_3N; *d*: Ac_2O/Py/DMAP, 16 h; *e*: 70% HF/Py, 1eq Ac_2O, 24 h; *f*: $Me_3SiOMu/BF_3 \cdot Et_2O/CH_2Cl_2$, 24 h; *g*: MeONa/MeOH; *h*: $NaOH/H_2O$; *i*: Ac_2O/Py, 60°C, 16 h; *j*: MeONa/MeOH.

Scheme.

Apparently, more effective course of the reaction is promoted in this case by the circumstance that the 2,4,6-trimethylpyridinium fluoride melt is an ionic liquid unlike the KHF_2 solution in ethylene glycol or HF solution in dioxane. It has recently been shown that some other ionic liquids, e.g., reagent EMIMF $(HF)_{2.3}$, which is ethylmethylimidazolium fluoride containing from 2 to 3 mol hydrogen fluoride, can be effectively applied to the synthesis of hydrofluorides by opening alkylarylepoxides [16]. In this connection, the study of opening reactions of anhydrosugars in the medium of ionic liquids, HF donors, seems to be a prospective direction in the further research.

The structure of (**II**) follows from coincidence of its melting point and a close value of $[\alpha]_D$ in comparison with the known data [13]. Its ^{1}H NMR spectrum has a characteristic value of $J_{H4,F}$ constant equal to 50.1 Hz, and also spin coupling constants $J_{C3,F}$, $J_{C4,F}$, and $J_{C5,F}$ equal to 29.9, 182.0, and 22.0 Hz, respectively.

The further four-step transformation of compound (**II**) to product (**III**) (closure of 2,3-anhydrocycle, its 1,2-*trans*-diaxial opening by ammonia, *N*-trifluoroacetylation, and *O*-acetylation) was carried out without isolation of intermediate products. The point is that, during preliminary experiments, it has been established that the literature stage of 2,3-anhydrocycle closure in similar derivatives can be modified by applying a solution of ammonia in methanol instead of a solution sodium methylate in a methanol–chloroform mixture. After heating of (**II**) with a methanolic solution of ammonia, the formed amino group at C2 was transformed to trifluoroacetamide group by the action of methyl trifluoroacetate and free hydroxy group at C3 was converted into *O*-acetyl group by the action of acetic anhydride in pyridine. The acetylation of hydroxy group at C3 was confirmed by a downfield shift of ring H3 signal in ^{1}H NMR spectrum (δ 3.70 to 4.88 ppm) and an appearance of singlet in area of δ 2.14 ppm ($C\underline{H}_3COO$). The presence of amide group in product (**III**) follows from the disappearance of singlet signal at δ 2.44 ppm ($OS(O)_2C_6H_4C\underline{H}_3$) in the spectrum of ^{1}H NMR and an upfield shift of the signal corresponding to C2 (δ 77.0 ⟶ 49.1 ppm) in the spectrum of ^{13}C NMR, and also the appearance of a doublet at δ 6.75 ppm corresponding to amide NH ($N\underline{H}$ $J_{NH,2}$ 8.0 Hz).

Transformation of 1,6-anhydroderivative (**III**) to glycosyl fluoride (**IV**) was achieved by its treatment by 70% solution of hydrofluoric acid in pyridine (Py 9HF, Olah's reagent) with addition of 1 equiv of acetic anhydride. Note that two- to three-step sequence of reactions

of acetolysis and acetoxyl replacement of halogen atom or another leaving group at C1 is usually applied as the main way of transition from 1,6-anhydrosugars to glycosyl donors [12, 17, 18]. By the example of successful single step opening of compound (**III**), we managed to show that a direct transition from 1,6-anhydro derivatives to glycosyl fluorides is possible. The formation of glycosyl fluoride (**IV**) was confirmed by the appearance in the ^{1}H NMR spectrum of a new singlet at δ 2.13 ppm corresponding to acetyl group at C6, and also change of spin-coupling values of ring signals in the final spectrum characteristic of $^4C_1$ conformation of glucopyranose ring, which indicate the opening of 1,6-anhydro ring? The size of $J_{1,2}$ constant in the spectrum of a ^{1}H NMR (<1 Hz) of product (**IV**) allows the attribution of α-configuration to the product, though this constant is significantly less than that of triacetate of 4-fluoro-4-deoxy-α-glucopyranosyl fluoride (2.8 Hz) [14]. At the same time, the β-configuration of anomeric center should be excluded, because the spin-coupling constant $J_{1,2}$ of 4-fluoro-4-deoxy-β-glucopyranosyl fluoride triacetate is 5.8 Hz [14]. The fact of a sharp reduction of $J_{1,2}$ in the derivative (**IV**) in comparison with 2-*O*-acetyl analogue is curious, and it would be interesting to compare constants of other *N*-trifluoroacetyl α-glycosides with their *O*-acetyl analogues. The configuration of C4 in product (**IV**) follows from the appearance in the ^{1}H NMR spectrum of characteristic signal H4 as a doublet of triplets at δ 4.63 ppm with constants $J_{H,F}$ 50.2, $J_{4,5}$ 9.5, and $J_{4,3}$ 9.4 Hz.

The resulting glycosyl donor (**IV**) was transformed to glucosaminide (**V**) by interaction with Me_3SiMu in the presence of boron trifluoride etherate. Glycoside (**V**) was isolated in 28% yield. In addition to (**V**), formation of trace quantities of the corresponding α-anomer was noticed in the reaction according to ^{1}H and a ^{13}C NMR spectra. Formation of (**V**) was confirmed by the appearance in ^{1}H and ^{13}C NMR spectra of the signals corresponding to methyl group and aromatic part of Mu aglycone (see the Experimental section). The value of $J_{1,2}$ constant of 8.3 Hz in ^{1}H NMR spectrum indicates the formation of β-anomer.

A similar glycosylation reaction with the use of β-glycosyl fluorides as donors and 4-methyl-7-trimethylsilyloxycoumarin as acceptor has been described in [19] (yield of reaction 86%, β/α ~3.3/1; the donor/$BF_3 \cdot Et_2O$/Me_3SiOMu ratio of 1/1/1.1). The differences of glycosyl fluoride (**IV**) from the analogue described in literature are the presence of fluorine atom at C4 instead of acetyl group and α-configuration of the anomer center. Now it is impossible to reliably estimate the contributions of each of these factors into a lowered reactivity of (**IV**) in comparison with 4-*O*-acetyl analogue. All the attempts to optimize the reaction conditions have not led to any increase in yield, which was 28% at a ratio donor/$BF_3 \cdot Et_2O$/Me_3SiOMu 1/2/1.1.

Further transformations *g–j* (scheme) of glycoside (**V**) led to completely acetylated derivative (**VI**), the structure of which was proved by the appearance of new signals in ^{1}H- and ^{13}C NMR spectra at δ 1.98 (s) and 23.3 ppm, respectively. The further transformation of compound (**VI**) to the target glucosaminide (**VII**) was carried out. There were no signals from two *O*-acetyl groups in ^{1}H NMR spectrum of product (**VII**) and strong upfield shifts of protons H3 (δ 5.48 ⟶ 3.98 ppm) and H6 (δ 4.48–4.47 ⟶ 3.73–3.94 ppm) were observed.

The resulting compounds (**III**), (**VI**) and (**VII**) were additionally characterized with the use of spectral techniques COSY-^{1}H-^{1}H and ^{1}H-^{13}C, HSQC-^{1}H-^{1}H and ^{1}H-^{13}C. This also allowed the assignment of signals for analogues of these compounds, derivatives (**IV**) and (**V**). These assignments do not leave any doubts concerning the structure of the compounds synthesized.

Thus, our work opens in an efficient way to 4-deoxy-4-fluoro derivatives of levoglucosan. A transition from them to glucosaminide glycosyl fluoride was developed. By the example of synthesis of a fluorogenic substrate of β-hexosaminidase containing a fluorine atom in position 4 of the *N*-acetylglucosamine residue, the synthetic potential of 4-deoxy-4-fluoroglucosaminide donors in glycoside synthesis was revealed.

EXPERIMENTAL

We used in the work reagents of firms Fluka, Acros, and Merck. Column chromatography was carried out on silica gel 60 (Merck) and Silpearl; TLC, on aluminium and plastic plates from Merck and Silufol with and without an UV-indicator in systems ethyl acetate–toluene (EA : Tol), toluene–ether (Tol : Et_2O) and ethyl acetate–ethanol (EA : EtOH). Detection of substance spots on TLC plates was achieved by heating (up to ~150°C) or, at the use of Merck plates, by a preliminary treatment with 10% H_3PO_4 solution in EtOH. Angles of optical rotation were measured on a Jasco DIP-360 polarimeter at 20–25°C. Spectra of ^{1}H and ^{13}C NMR were registered on Bruker AC200, AM300, and WM250 instruments for solutions of substances in $CDCl_3$ (δ, ppm; spin-coupling costants, Hz), chemical shifts are given relative Me_4Si. All experiments on bidimentional correlation spectroscopy were carried out by standard techniques of the Bruker firm.

1,6-Anhydro-4-deoxy-2-*O*-tosyl-4-fluoro-β-*D*-glucopyranose (II). Compound (**I**) [11] (5.00 g, (16.7 mmol) and collidinium hydrofluoride (27.00 g, 0.19 mol) (Fluka) were placed in an autoclave with a tetrafluoroethylene test-tube and heated for 55 h at 125°C. The reaction mixture was then cooled, diluted with 300 ml of chloroform and washed with distilled water (~500 ml). The organic layer was separated, dried with anhydrous Na_2SO_4, and evaporated. TLC (system EA–Tol, 1 : 4) showed that the resulting dark orange residue contained the reaction product (R_f 0.34) and the starting compound (R_f 0.63) at a ratio of ~1 : 1. A chromatography of the mixture on silica gel in system 1 : 5

EA–Tol allowed the separation of starting compound (**I**) (2.04 g) and 4-fluoro derivative (**II**), which was recrystallized from benzene; yield 2.50 g (47%), colorless crystals; R_f 0.34 (EA–Tol, 1 : 4); mp 117–118°C (mp (lit) 115–116°C [13, 14]), $[\alpha]_D$ –39° (*c* 1, chloroform); ^{1}H NMR: 2.44 (3 H, s, C$\underline{H}_3$), 2.89 (1 H, br. s, OH), 3.70 (1 H, m, H3), 3.97–4.08 (2 H, m, H6a, H6b), 4.23 (1 H, s, H2), 4.41 (1 H, d, $J_{H4,F}$ 50.1, H4), 4.71 (1 H, dd, H5), 5.33 (1 H, s, H1), 7.35–7.83 (4 H, two d, OTos); ^{13}C NMR: 21.7 (s, $\underline{C}H_3$), 64.8 (d, $J_{C6,F}$ 14.3, C6), 69.5 (d, $J_{C3,F}$ 29.9, C3), 74.12 (d, $J_{C5,F}$ 22.0, C5), 77.0 (s, C2), 89.8 (d, $J_{C4,F}$ 182.0, C4), 99.6 (s, C1).

1,6-Anhydro-3-*O*-acetyl-2,4-dideoxy-2-trifluoroacetamido-4-fluoro-β-*D*-glucopyranose (III). Compound (**II**) (1.25 g, 3.79 mmol) and 17 ml of MeOH were placed in a glass ampoule, the mixture was saturated with ammonia at –15°C, and the ampoule was sealed. The reaction mixture was kept at 105°C for 15 h, then the ampoule was opened, and its content was evaporated. Metyl trifluoroacetate (1 ml), MeOH (5 ml), and triethylamine (0.5 ml) were added to the yellow residue, and the mixture was kept for 16 h at 20°C and evaporated. The residue was dissolved in pyridine (15 ml), Ac_2O (1.5 ml) and DMAP (10 mg) were added to the solution, and the mixture was kept for 16 h. Water (50 ml) was further added to the mixture, and it was coevaporated with toluene (3 × 20 ml). The residue was chromatographed on a column in 1 : 10 EA–Tol system; compound (**III**) (1.05 g, 90%) was obtained as colorless oil; R_f 0.43 (Et_2O : Tol, 1 : 1); $[\alpha]_D$ –58° (*c* 1, chloroform); ^{1}H NMR: 2.14 (3 H, s, COC$\underline{H}_3$), 3.87 (1 H, m, H6a), 4.09–4.11 (2 H, m, H6b, H2), 4.46 (1 H, d, $J_{H4,F}$ 43.5, H4), 4.76 (1 H, t, H5), 4.88 (1 H, d, H3), 5.43 (1 H, s, H1), 6.75 (1 H, d, $J_{NH,H2}$ 8.0, N$\underline{H}$); ^{13}C NMR: 20.7 (s, CO$\underline{C}H_3$), 49.1 (s, C2), 64.3 (d, C6), 68.9 (d, C3, $J_{C3,F}$ 32.4), 73.6 (d, C5, $J_{C5,F}$ 20.2), 86.6 (d, C4, $J_{C4,F}$ 180.0), 99.7 (s, C1).

3,6-Di-*O*-acetyl-2,4-dideoxy-2-trifluoroacetamido-4-fluoro-α-*D*-glucopyranosyl fluoride (IV). Compound (**III**) (1.07 g, 3.59 mmol), 15 ml of 70% HF in pyridine, and 0.5 ml of Ac_2O were placed in a tetrafluoroethylene vessel, and kept for 24 h at 20°C. The reaction mixture was diluted with dichloromethane (50 ml) and poured at intensive stirring in 10-ml portions in a glass with a saturated solution of $NaHCO_3$ (300 ml) with ice. The organic layer was separated, dried with anhydrous Na_2SO_4, and evaporated. Crystallization from toluene gave 0.82 g (63%) of colorless crystals of glycosyl fluoride (**IV**); R_f 0.44 (Et_2O : Tol, 1 : 3), mp 114–115°C, $[\alpha]_D$ + 2° (*c* 1, chloroform); ^{1}H NMR: 2.13 (6 H, s, 2 × COC$\underline{H}_3$), 4.22 (1 H, m, H5), 4.28–4.34 (2 H, m, H6a, H2), 4.63 (1 H, dt, $J_{H4,F}$ 50.2, $J_{H4,H5}$ 9.5, $J_{H4,H3}$ 9.4, H4), 4.48 (1 H, d, $J_{H6a,H6b}$ 12.5, H6b), 5.46 (1 H, m, $J_{H3,F}$ 12.3, $J_{H3,H4}$ 10.3, H3), 5.67 (1 H, d, $J_{H1,F}$ 52.2, H1), 7.05 (1 H, d, $J_{H2,NH}$ 8.1, N$\underline{H}$); ^{13}C NMR: 20.5 (s, CO$\underline{C}H_3$), 20.7 (s, CO$\underline{C}H_3$), 49.1 (s, C2), 61.3 (s, C6), 69.5–70.1 (m, C3, C5), 85.4 (d, $J_{C4,F}$ 188.1, C4), 104.8 (d, $J_{C1,F}$ 226.1, C1). MS, *m*/*z*: 344 (363–19) ($M^+ - F^-$).

Methylumbelliferyl 3,6-di-*O*-acetyl-2,4-dideoxy-2-trifluoroacetamido-4-fluoro-β-*D*-glucopyranoside (V). Glycosyl fluoride (**IV**) (0.40 g, 1.1 mmol) and 4-methyl-7-trimethylsilyloxycoumarin [17] (0.30 g, 1.2 mmol) in 2 ml of dry dichloromethane were added dropwise for 30 min to $BF_3 \cdot Et_2O$ (0.3 ml, 2.1 mmol) in 1 ml of dichloromethane under vigorous stirring. After 24 h, dichloromethane (20 ml) was added to the reaction mixture; it was washed with water (2 × 30 ml), and organic layer was filtered and evaporated. The residue was chromatographed in 2.5 : 1 Tol–Et_2O system and the isolated product was recrystallyzed from ethanol to get 0.16 g (28%) of (**V**) as colorless crystals; R_f 0.4 (1 : 1 Et_2O–Tol.), mp 178–179°C. ^{1}H NMR: 2.14 (3 H, s, COC$\underline{H}_3$), 2.16 (3 H, s, COC$\underline{H}_3$), 2.37 (3 H, s, C$\underline{H}_3$), 4.02 (1 H, m, H5), 4.31 (1 H, dd, $J_{H6a,H5}$ 5.5, $J_{H6a,H6b}$ 12.2, H6a), 4.43 (1 H, q, $J_{H2,NH}$ 9.6, $J_{H2,H3}$ 9.2, H2), 4.51 (1 H, d, $J_{H6a,H6b}$ 12.3, H6b), 4.63 (1 H, dt, $J_{H4,F}$ 50.5, $J_{H4,H3}$ 9.1, $J_{H4,H5}$ 9.2, H4), 5.41 (1 H, d, $J_{H1,H2}$ 8.3, H1), 5.57 (1 H, m, H3), 6.12 (1 H, s, H3'), 6.90 (1 H, dd, $J_{H6',H5'}$ 8.8, $J_{H6',H8'}$ 2.15, H6'), 6.92 (1 H, d, $J_{H8',H6'}$ 2.1, H8'), 7.48 (1 H, d, $J_{H5',H6'}$ 8.75, H5'), 7.62 (1 H, d, $J_{NH,H2}$ 9.3, N$\underline{H}$); ^{13}C NMR: 18.6 (s, CO$\underline{C}H_3$), 20.6 (s, CO$\underline{C}H_3$), 53.9 (d, $J_{C2,F}$ 7.1, C2), 61.9 (s, C6), 71.2 (d, $J_{C3,F}$ 22.8, C3), 71.8 (m, C5), 86.6 (d, $J_{C4,F}$ 188.2, C4), 98.0 (s, C1), 103.4 (s, C6'), 112.9 (s, C3'), 114.7 (s, C8'), 125.8 (s, C5').

Methylumbelliferyl *N*-acetyl-3,6-di-*O*-acetyl-4-deoxy-4-fluoro-β-*D*-glucosaminide (VI). A solution of MeONa/MeOH (3 ml MeOH, 6 mg Na) was added to (**V**) (80 mg, 150 μmol), and the mixture was kept for 16 h at room temperature. Then methyl trifluoroacetate (0.3 ml) was added. The mixture was kept for 1.5 h, neutralized with cation exchanger Dowex 50WX2 in H^+-form up to pH 7, the solution was filtered and evaporated. Acetone (1.5 ml) and NaOH (27 mg in 1.5 ml of water) were added to the reaction mixture, it was kept for 1 h at room temperature, excess of NaOH was neutralized with 0.2 ml of AcOH, and the reaction mixture was evaporated. An Ac_2O/pyridine (0.5 ml + 0.5 ml) mixture was added to the residue, kept for 24 h at room temperature, diluted with water (30 ml), and evaporated. The chromatography of the residue in ethyl acetate and the subsequent crystallization from isopropanol resulted in product (**VI**); yield 35 mg (50%); colorless crystals; R_f 0.48 (EA), mp 270–272°C, $[\alpha]_D$ –34° (*c* 1, chloroform); ^{1}H NMR: 1.98 (3 H, s, NCOC$\underline{H}_3$), 2.14 (3 H, s, COC$\underline{H}_3$), 2.16 (3 H, s, COC$\underline{H}_3$), 2.39 (3 H, s, C$\underline{H}_3$), 3.98 (1 H, m, H5), 4.28 (2 H, m, H6a, H2), 4.47 (1 H, d, $J_{H6a,H6b}$ 12.2, H6b), 4.57 (1 H, dt, $J_{H4,F}$ 50.6, $J_{H4,H3}$ 9.1, $J_{H4,H5}$ 9.2, H4), 5.32 (1 H, d, $J_{H1,H2}$ 8.2, H1), 5.48 (1 H, m, H3), 6.00 (1 H, d, $J_{NH,H2}$ 9.0, N$\underline{H}$), 6.17 (1 H, s, H3'), 6.94 (2 H, m, H6', H8'), 7.50 (1 H, d, $J_{H5',H6'}$ 9.42, H5'); ^{13}C NMR: 18.6 (s, $\underline{C}H_3$), 20.7 (2 s, CO$\underline{C}H_3$), 23.2 (s, NCO$\underline{C}H_3$), 53.9 (s, C2), 62.0 (s, C6), 71.6–72.0 (m, C3, C5), 86.7 (d, C4, $J_{C4,F}$

187.1), 98.4 (s, C1), 103.9 (s, C6'), 113.0 (s, C3'), 114.0 (s, C8'), 125.7 (s, C5').

Methylumbelliferyl *N*-acetyl-4-deoxy-4-fluoro-β-*D*-gglucosaminide (VII). A solution of sodium methylate (0.2 ml, 11 mg Na, 10 ml MeOH) was added to a suspension of (**VI**) (8.7 mg, (19 μmol). The mixture was kept for 16 h at room temperature, neutralized with cation exchanger Dowex 50WX2 (H^+-form), filtered, and evaporated. The obtained oil was dissolved in water and lyophilized. Compound (**VII**) (6.4 mg, 90 %) was obtained as a colorless powder; R_f 0.49 (EA–EtOH, 5 : 1); mp 243–244°C, $[\alpha]_D$–7° (*c* 0.5, methanol); ^{1}H NMR: 1.99 (3 H, s, C$\underline{H}_3$), 2.43 (3 H, s, NCOC$\underline{H}_3$), 3.73–3.79 (2 H, m, H6a, H5), 3.88–3.94 (1 H, m, H6b), 3.96–3.99 (2 H, m, H2, H3), 4.39 (1 H, dt, $J_{H4,F}$ 50.6, $J_{H4,H3}$ 8.4, $J_{H4,H5}$ 8.4, H4), 5.28 (1 H, d, $J_{H1,H2}$ 8.0, H1), 6.22 (1 H, s, H3'), 7.00–7.04 (2 H, m, H6', H8'), 7.72 (1 H, d, $J_{H5',H6'}$ 9.42, H5'); ^{13}C NMR: 18.6 (s, $\underline{C}H_3$), 22.9 (s, NCO$\underline{C}H_3$), 57.0 (d, $J_{C2,F}$ 7.1, C2), 61.5 (s, C6), 73.2 (d, $J_{C3,F}$ 18.8, C3), 75.6 (d, $J_{C5,F}$ 25.0, C5), 90.6 (d, $J_{C4,F}$ 181.2, C4), 99.9 (s, C1), 104.8 (s, C6'), 113.0 (s, C3'), 115.0 (s, C8'), 127.4 (s, C5').

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REFERENCES

1. Tsuchiya, T., *Adv. Carbohydr. Chem. Biochem.*, 1990, vol. 48, pp. 91–277.
2. Dax, K., Albert, M., Ortner, J., and Paul, B., *Carbohydr. Res.*, 2000, vol. 327, pp. 47–86.
3. Card, J., *J. Carbohydr. Chem*, 1985, vol. 4, pp. 451–487.
4. Thomas, R., Abbas, S., and Matta, K., *Carbohydr. Res.*, 1988, vol. 175, pp. 153–157.
5. Kajihara, Y., Hasashi, K., Tsuyoshi, E., and Hironobu, H., *Carbohydr. Res.*, 1998, vol. 306, pp. 361–378.
6. Taichi, U., Fei, F., Reiko, S., Kenichi, N., Kenji, M., and Shin-Ichiro, N., *Org. Lett.*, 2004, vol. 6, pp. 1753–1756.
7. Chapeau, M. and Frey, P., *J. Org. Chem.*, 1994, vol. 59, pp. 6994–6998.
8. Berkin, A., Szarek, W., and Kisilevsky, R., *Carbohydr. Res.*, 2000, vol. 326, pp. 250–263.
9. Foster, A., Hems, R., and Westwood, J., *Carbohydr. Res.*, 1970, vol. 15, pp. 41–49.
10. Hough, L., Penglis, A., and Richardson, A., *Can. J. Chem.*, 1981, vol. 59, pp. 396–405.
11. Černý, M., Gut, V., and Pacák, J., *Collect. Czech. Chem. Commun.*, 1961, vol. 26, pp. 2542–2550.
12. Pacák, J., Podešva, J., Točik, Z., and Černý, M., *Collect. Czech. Chem. Commun.,* 1972, vol. 37, pp. 2589–2599.
13. Pacák, J., Drašar, P., Nerudová, J., and Černý, M., *Collect. Czech. Chem. Commun.*, 1972, vol. 37, pp. 4120–4125.
14. Barford, A., Foster, A., Westwood, J., Hall, L., and Johnson, R., *Carbohydr. Res.*, 1971, vol. 19, pp. 49–61.
15. Haufe, G., *J. Fluorine Chem.,* 2004, vol. 125, pp. 875–894.
16. Yoshino, H., Matsumoto, K., Hagiwara, R., Ito, Y., Oshima, K., and Matsubara, S., *J. Fluorine Chem.*, 2006, vol. 127, pp. 29–35.
17. Dasgupta, F. and Masada, R., *Carbohydr. Res.*, 2002, vol. 337, pp. 1055–1058.
18. Bochkov, A. and Voznyi, Ya., *Carbohydr. Res.*, 1974, vol. 32, pp. 1–8.
19. Voznyi, Ya., Afanas'eva, S., Kalicheva, I., and Galoyan, A., *Bioorg. Khim.*, 1991, vol. 17, pp. 1251–1257.