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Mendeleev Commun., 2015, **25**, 422–423
Mendeleev Communications

Synthesis of 1-BODIPY-labeled 2-amino-2-deoxy-D-glucose, substrate for acetyl-CoA:glucosaminide *N*-acetyltransferase

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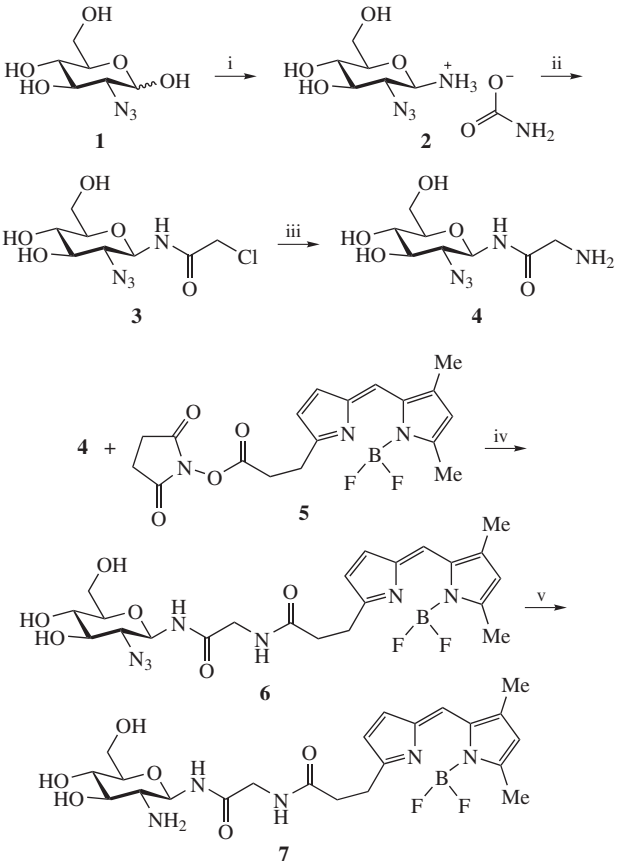
DOI: 10.1016/j.mencom.2015.11.007

A new fluorescent substrate for acetyl-CoA: glucosaminide *N*-acetyltransferase, namely, 2-amino-2-deoxy-D-glucose BODIPY-labeled by the 1-position, was synthesized in five steps from 2-azido-2-deoxy-D-glucose.

In humans the genetic deficiency of heparan sulfate (HS) acetyl-CoA: α-glucosaminide *N*-acetyltransferase (HGSNAT), which catalyzes acetylation of HS prior to its hydrolysis, causes mucopolysaccharidosis III type C (Sanfilippo disease), progressive neurodegeneration disease of children, leading to dementia and death before adulthood.[1] Patients can potentially benefit from a therapeutic approach based on active site-specific inhibitors of HGSNAT used as pharmacological chaperons to modify the folding of the mutant protein in the patient's cells. However, this research is hampered by the absence of the assay suitable for high-throughput screening of drug candidates.[2] The existing method with 4-methylumbelliferyl-β-D-glucosaminide requires the sequential action of two enzymes, HGSNAT and β-hexosaminidase,[3] whereas the radioactive assay with [^{14}C]-AcCoA is complicated and expensive. The aim of this work was to synthesize new substrate for HGSNAT, namely, 2-amino-2-deoxy-D-glucose fluorescent-labeled by the 1-position starting from 2-azido-2-deoxy-D-glucose.

Synthesis of BODIPY-labeled 3-aminopropyl glycosides of several sialooligosaccharides as well as their application for the study of influenza virus neuraminidases were previously reported.[4,5] The cited works demonstrated that C_3-distancing (aminopropyl spacer) is enough to avoid interference of the label with enzyme/substrate interaction, and that BODIPY does not cause an unspecific interaction with neuraminidases. Particular commercially available reagent BODIPY® FL NHS ester **5** has been selected because BODIPY® FL is similar to regular fluorescein by its exitation/emission characteristics, and has no charge. This is important for analytical stage of ion-exchange based separation of positively charged substrate and neutral (due to acylation of free NH_2 group) product of enzymatic reaction. Here we describe the synthesis of fluorescent BODIPY-labeled *N*-glycylglycosylamine derivative of 2-amino-2-deoxy-D-glucose.

Starting from 2-azido-2-deoxy-D-glucose **1**, *N*-glycylglycosylamine derivative **4** was synthesized *via* glycosylamine carbamate **2** (by analogy with procedure reported for GlcNAc),[6] *N*-chloroacetamide **3** and its consecutive ammonolysis (the procedures of N-chloroacetylation of glycosylamines and ammonolysis[7] were modified as described[8]) (Scheme 1). Obtained *N*-glycylglycosylamine **4** was isolated by ion-exchange chromatography (yield 65%) followed by crystallization from MeOH–Pr^{i}OH to remove admixtures of α-anomer and product with *manno* configuration.†



Scheme 1 *Reagents and conditions*: i, $H_2NCOONH_4$, MeOH/H_2O, 42 h at 40 °C; ii, $(ClCH_2CO)_2O$/$NaHCO_3$, toluene/H_2O; iii, NH_3 aq.; iv, DMSO; v, PPh_3, THF/H_2O.

2-Azido-2-deoxy-Glcβ-NH(CO)CH$_2$NH$_2$ **4**. Compound **1** (294 mg, 1.43 mmol) and ammonium carbamate (447 mg, 5.73 mmol) were dissolved in a mixture of methanol (3.6 ml) and water (0.036 ml), kept for 42 h at 40 °C and for 15 h at 4 °C (not crystallized), diluted with water (1 ml), evaporated and dried *in vacuo*. Freeze-drying from water gave **2** (351 mg, 92%) that was used without purification. A solution of $(ClCH_2CO)_2O$ (980 mg, 5.73 mmol) in toluene (5 ml) was added to a solution of **2** (351 mg) in 1 M aqueous $NaHCO_3$ (10.1 ml) at 10 °C. The mixture was stirred for 30 min at room temperature, acidified with AcOH (164 μl), evaporated and dried. The residue was dissolved in water (1 ml), 10 M aqueous NH_3 (20 ml) was added and the solution was kept for 24 h at room temperature. The solution was evaporated, dried, the residue was dissolved in water (3 ml), acidified with AcOH (300 μl) and the solution

† ^{1}H NMR spectra, MALDI TOF mass spectra and TLC data of all synthesized compounds are available in Online Supplementary Materials.

Condensation of *N*-glycylglycosylamine derivative **4** with 1 equiv. of succinimidyl ester of BODIPY® FL **5** was carried out as described for 3-aminopropyl glycosides[5] in 76% yield.‡

For reduction of azido group, triphenylphosphine was used. Since efficacy of reduction of 2-azido sugars is strictly structure-dependent, we examined different conditions. It was found that THF/H_2O or MeOH/H_2O are suitable as solvents, and the reaction was completed with the use of 5-fold excess of triphenylphosphine. Amino compound **7** was isolated by gel-chromatography followed by silica gel chromatography. Pure product **7** (>98%) was obtained as AcOH-salt in 50% yield.§

The specificity of substrate **7** based assay was tested using cultured fibroblasts of MPS IIIC patients, which showed a profound deficiency of HGSNAT activity as compared to normal controls. Known competitive HGSNAT inhibitor, glucosamine, had similar inhibition constants for MU-βGlcN and BODIPY-glucosamine acetylation reactions. The data show that novel substrate is specific and potentially applicable for the biochemical diagnosis of MPS IIIC and high-throughput screening for HGSNAT inhibitors.¶

This work was supported by the Russian Science Foundation (project no. 14-14-00579).

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2015.11.007.

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Received: 8th April 2015; Com. 15/4599

was kept for 16 h at room temperature. Products were separated by ion-exchange chromatography on Dowex 50X4-200 (H^+), elution with water followed by 1 M aqueous pyridine to remove acids and glycine. Elution with 1 M aqueous NH_3 gave 243 mg (65%) of material containing minor admixtures. Crystallization from MeOH/Pr^{i}OH afforded 105 mg of pure colorless **4**.

‡ *2-Azido-2-deoxy-Glcβ-NH(CO)CH_2NH-BODIPY* **6**. Derivative **4** (6.7 mg, 0.026 mmol) was added to a solution of compound **5** (10 mg, 0.026 mmol) in DMSO (1 ml). The reaction mixture was kept at 20 °C for 5 min and the product was isolated on Sephadex LH-20. Additional purification on silica gel yielded 10.5 mg (76%) of pure **6**.

§ *2-Amino-2-deoxy-Glcβ-NH(CO)CH_2NH-BODIPY* **7**. A solution of PPh_3 (18.4 mg, 0.07 mmol) in THF/H_2O (3:1 v/v, 1.8 ml) was added to a solution of compound **6** (7.5 mg, 0.014 mmol) in the same solvent (1.5 ml) and stirred overnight at 20 °C. The solution was concentrated, the residue was dissolved in H_2O (2 ml), and the excess of PPh_3 was removed by extraction with toluene. Gel-chromatography on Sephadex LH-20 followed by silica gel (CH_2Cl_2/EtOH/H_2O, 6:5:1 v/v) and additional gel-chromatography on Sephadex LH-20 gave pure amino compound **7** as AcOH-salt in 50% yield, red powder stable for keeping at –18 °C (dried or in water solution), unstable in DMSO. TLC, R_f = 0.4 (CH_2Cl_2/EtOH/H_2O, 6:5:1 v/v). ^{1}H NMR (700 MHz, D_2O) δ: 1.937 (s, 3H, AcOH), 2.214 (s, 3H, Me of BODIPY), 2.493 (s, 3H, Me of BODIPY), 2.784 (t, 2H, CH_2 of BODIPY, J 7.4 Hz), 3.004 (t, 1H, H-2, $J_{1,2} \approx J_{2,3} \approx 9.9$ Hz), 3.191 (t, 2H, $COCH_2$ of BODIPY, J 7.4 Hz), 3.423 (t, 1H, H-4, $J_{3,4} \approx J_{4,5} \approx 9.5$ Hz), 3.549 (m, 1H, H-5), 3.631 (t, 1H, H-3, $J_{2,3} \approx J_{3,4} \approx 9.5$ Hz), 3.717 (dd, 1H, H-6a, $J_{5,6a}$ 5.4 Hz, $J_{6a,6b}$ 12.4 Hz), 3.877 (dd, 1H, H-6b, $J_{5,6b}$ 1.6 Hz, $J_{6a,6b}$ 12.4 Hz), 3.959 (d, 1H, COCHN, $J_{H,H'}$ 17.1 Hz), 4.014 (d, 1H, COCH′N, $J_{H,H'}$ 17.1 Hz), 5.217 (d, 1H, H-1, $J_{1,2}$ 9.7 Hz), 6.273 (s, 1H, BODIPY), 6.379 (d, 1H, BODIPY, J 4.0 Hz), 7.009 (d, 1H, BODIPY, J 4.0 Hz), 7.368 (s, 1H, BODIPY). MS (MALDI-TOF), *m/z*: 510 [M+H], 532 [M+Na], 548 [M+K] (calc. for $C_{22}H_{30}BF_2N_5O_6$, M_w = 509.32).

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