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

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Starting from 2-azido-2-deoxy-D-glucose **1** *N*-glycylglycosylamine derivative **4** was synthesized via glycosylamine carbamate **2** (by analogy of procedure described for GlcNAc)[1], *N*-chloroacetamide **3** and its consecutive ammonolysis (the procedures of *N*-chloroacetylation of glycosylamines and ammonolysis[2] were modified as described earlier[3]). The obtained *N*-glycylglycosylamine **4** was isolated by ion-exchange chromatography (yield 65%) followed by crystallization from MeOH/2-propanol to remove admixtures of α-anomer and product with *manno* configuration.
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[4] in 76% yield.

2-Azido-2-deoxy-D-glucose **1** was purchased from Carbosynth Ltd (Compton Berkshire, UK), succinimidyl ester of 4.4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionic acid (BODIPY® FL NHS Ester) **5** was from Invitrogen (USA). Reactions were performed with the use of commercial reagents (Acros, Aldrich and Fluka). Column chromatography was performed on Silica gel 60 0.040–0.063 mm (Merck), gel-permeation chromatography was carried out on Sephadex LH-20 (GE Healthcare, Sweden) columns. Solvents were removed by evaporation in vacuum at 30-40 °C. Thin-layer chromatography (TLC) was performed on Silica gel 60 F_{254} aluminium sheets (Merck). Spots of compounds were visualized via either heating with H_3PO_4 (8%) or ninhidryn. ^{1}H NMR spectra were recorded on a Bruker BioSpin GmbH (700 MHz) spectrometer at 30 °C: chemical shifts (δ, ppm) were referred to the peak of internal D_2O (δ 4.750 ppm), coupling constants (J) were measured in Hz. MALDI TOF mass-spectra were recorded on Bruker Daltonics Ultraflex MALDI TOF/TOF Mass Spectrometer (Germany), DHB matrix.

*2-Deoxy-2-azido-Glcβ-NH(CO)CH_2NH_2 (**4**).*

2-Deoxy-2-azido-Glc **1** (294 mg, 1.433 mmol) and powdered ammonium carbamate (447 mg, 5.726 mmol) were dissolved in a mixture of methanol (3.6 ml) and water (0.036 ml) at 40 °C. The solution was kept for 42 h at 40 °C, cooled to room temperature and then was kept for 15 h at 4 °C: formation of crystalline glycosylamine carbamate was not observed. The solution was diluted with water (1 ml), evaporated and dried in vacuum. The residue was dissolved in water (9 ml) and freeze-dried (at 0.002 mBar for 16 h): weight of a residue ~ corresponded to glycosylamine carbamate **2** (351 mg, 92%).

To an intensively stirred solution of 2-deoxy-2-azido-Glc glycosylamine carbamate **2** (351 mg) in cooled (10 °C) 1 M aqueous $NaHCO_3$ (10.1 ml) a solution of chloroacetic acid anhydride (980 mg, 5.732 mmol) in toluene (5 ml) was added. The mixture was stirred for 30 min at room temperature, acidified with AcOH (164 μl), evaporated and dried.

The residue (consisted of *N*-chloroacetamide **3** and sodium chloroacetate) 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, a residue dissolved in water (3 ml), acidified with AcOH (300 μl) and the solution was kept for 16 h at room temperature. Products were separated by ion-exchange chromatography on Dowex 50X4-200 (H^+) column (40 ml). Elution with water (40 ml) and then with 1 M aqueous pyridine (70 ml) removed all acidic, neutral components and amino acids (including glycine formed from excessive chloroacetate). Elution with 1 M aqueous NH_3 (75 ml) gave 243 mg (65%) of material consisted of target 2-deoxy-2-azido-Glcβ-NH(CO)CH_2NH_2 **4** (90%), 2-deoxy-2-azido-Glcα-NH(CO)CH_2NH_2 (5%) and 2-deoxy-2-azido-Manβ-NH(CO)CH_2NH_2 (5%) by ^{1}H NMR data. Crystallization from MeOH/2-propanol gave slightly yellow crystals of 2-deoxy-2-azido-Glcβ-NH(CO)CH_2NH_2. Gel-permeation chromatography on Sephadex G-10 (90 ml) in MeOH/0.1 M aqueous NH_3 (1:3 by volume) and freeze-drying gave 105 mg of pure colorless **4**.

TLC: R_f = 0.47 (2-propanol/MeOH/1 M aqueous Py•HOAc 2:3:1 v/v).

^{1}H NMR of TFA-salt (700 MHz, D_2O) δ, ppm: 3.482 (t, 1H, $J_{1,2} \approx J_{2,3} \approx$9.6 Hz; H-2), 3.503 (t, 1H, $J_{3,4} \approx J_{4,5} \approx$9.5 Hz; H-4), 3.561 (ddd, 1H, $J_{4,5}$ = 9.7, $J_{5,6a}$ = 5.3, $J_{5,6b}$ = 1.9 Hz; H-5), 3.672 (t, 1H, $J_{2,3} \approx J_{3,4} \approx$9.4 Hz; H-3), 3.753 (dd, 1H, $J_{6a,6b}$ = 12.5, $J_{5,6a}$ 5.3 Hz; H-6a), 3.897 (dd, 1H, $J_{6a,6b}$ = 12.5, $J_{5,6b}$ 1.9 Hz; H-6b), 3.918 (d, 1H, $J_{H,H`}$ = 16.4 Hz; COCHN), 3.948 (d, 1H, $J_{H,H`}$ = 16.4 Hz; COCH`N), 5.073 (d, 1H, $J_{1,2}$ = 9.7 Hz; H-1).

MALDI TOF MS, *m/z*: 262 [M+H], 284 [M+Na], 300 [M+K] (calc. for $C_8H_{15}N_5O_5$, Mw = 261.24).

*2-Deoxy-2-azido-Glcβ-NH(CO)CH_2NH-BODIPY **6**.*

N-glycylglycosylamine derivative of 2-azidoglucose **4** (6.7 mg, 0.026 mmol) was added to a solution of BODIPY® FL NHS Ester **5** (10 mg, 0.026 mmol) in DMSO (1 ml). The reaction mixture was kept for 5 min at 20 °C and the product was isolated on Sephadex LH-20 (CH_3CN/H_2O 1:1 v/v). Additional purification on silica gel (CH_2Cl_2/EtOH 8:1→ 6:1 v/v) yielded 10.5 mg (76%) of BODIPY derivative **6**.

TLC: R_f = 0.4 (CH_2Cl_2/EtOH 6:1 v/v).

^{1}H NMR (700 MHz, D_2O) δ, ppm: 2.267 (s, 3H; CH_3 of BODIPY), 2.530 (s, 3H; CH_3 of BODIPY) 2.806 (t, 2H, J = 7.2 Hz; CH_2 of BODIPY), 3.235 (t, 2H, J = 7.3 Hz; $COCH_2$ of BODIPY), 3.326 (t, 1H, $J_{1,2} \approx J_{2,3} \approx$ 9.7 Hz; H-2), 3.365 (t, 1H, $J_{3,4} \approx J_{4,5} \approx$ 9.5 Hz; H-4), 3.485 (m, 1H; H-5), 3.577 (t, 1H, $J_{2,3} \approx J_{3,4} \approx$ 9.4 Hz; H-3), 3.674 (dd,1H, $J_{5,6a}$ = 5.7, $J_{6a,6b}$ = 12.4 Hz; H-6a), 3.861 (dd, 1H, $J_{5,6b}$ = 1.9, $J_{6a,6b}$ = 12.4 Hz; H-6b), 3.954 (d, 1H, $J_{H,H`}$ = 17.4 Hz; COCHN), 3.981 (d, 1H, $J_{H,H`}$ = 17.4 Hz; COCH`N), 4.968 (d, 1H, $J_{1,2}$ = 9.5 Hz; H-1), 6.324 (s, 1H; BODIPY), 6.432 (d, 1H, J = 4.0 Hz; BODIPY), 7.078 (d, 1H, J = 3.7 Hz; BODIPY), 7.463 (s, 1H; BODIPY).

MALDI TOF MS, *m/z*: 558 [M+Na], 574 [M+K] (calc. for $C_{22}H_{28}BF_2N_7O_6$, Mw = 535.32). Fragmentation: 488 [M-BF2+2H], 498 [M-2F+H], 510 [M-N_2+2H+H], 516 [M-F^-], 532 [M-N_2+2H+Na], 548 [M-N_2+2H+K].

*2-Deoxy-2-amino-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 azido-compound **6** (7.5 mg, 0.014 mmol) of in the same solvent (1.5 ml) and stirred overnight at 20 °C. The solution was concentrated in vacuum, the residue was dissolved in 2 ml H_2O, and the excess of triphenylphosphine was removed by extraction with toluene (3 x 4 ml). Gel-chromatography on Sephadex LH-20 (CH_3CN/H_2O 1:1, 0.05% AcOH) gave 5.6 mg (80%) of amino-compound **7** as AcOH salt. Further purification was carried out by subsequent column chromatography on silica gel (CH_2Cl_2/EtOH/H_2O 6:5:1) and additional gel-chromatography on Sephadex LH-20 (CH_3CN/H_2O 1:1, 0.02 M Py•HOAc). After freeze-drying pure amino-compound **7** was obtained 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, ppm: 1.937 (s, 3H; AcOH), 2.214 (s, 3H; CH_3 of BODIPY), 2.493 (s, 3H; CH_3 of BODIPY), 2.784 (t, 2H, J = 7.4 Hz; CH_2 of BODIPY), 3.004 (t, 1H, $J_{1,2} \approx J_{2,3} \approx$

9.9 Hz; H-2), 3.191 (t, 2H, J = 7.4 Hz; $COCH_2$ of BODIPY), 3.423 (t, 1H, $J_{3,4} \approx J_{4,5} \approx 9.5$ Hz; H-4), 3.549 (m, 1H; H-5), 3.631 (t, 1H, $J_{2,3} \approx J_{3,4} \approx 9.5$ Hz; H-3), 3.717 (dd,1H, $J_{5,6a} = 5.4$, $J_{6a,6b} = 12.4$ Hz; H-6a), 3.877 (dd, 1H, $J_{5,6b} = 1.6$, $J_{6a,6b} = 12.4$ Hz; H-6b), 3.959 (d, 1H, $J_{H,H`} = 17.1$ Hz; COCHN), 4.014 (d, 1H, $J_{H,H`} = 17.1$ Hz; COCH`N), 5.217 (d, 1H, $J_{1,2} = 9.7$ Hz; H-1), 6.273 (s, 1H; BODIPY), 6.379 (d, 1H, J = 4.0 Hz; BODIPY), 7.009 (d, 1H, J = 4.0 Hz; BODIPY), 7.368 (s, 1H; BODIPY).

MALDI TOF MS, *m/z*: 510 [M+H], 532 [M+Na], 548 [M+K] (calc. for $C_{22}H_{30}BF_2N_5O_6$, Mw = 509.32). Fragmentation: m/z 462 [M-BF2+H], 472 [M-2F+H], 490 [M-F^-].

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