SYNTHESIS OF NORMURAMIC ACID CARBA ANALOG AND ITS GLYCOPEPTIDE DERIVATIVE RESISTANT TO β-ELIMINATION SPLITTING OF THE SIDE CHAIN+

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Carba analogs of normuramic acid, *i.e.*, 3-(benzyl 2-acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-α-D-glucopyranosid-3-yl)propanoic acid derivatives (nitrile or esters) **3a**–**3c** were prepared by addition of radicals generated from benzyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[(methylsulfanyl)thiocarbonyl]- (**2a**) or -3-*O*-(phenoxythiocarbonyl)-α-D-glucopyranoside (**2b**) with Bu_3SnH to acrylonitrile or acryl esters. Alkaline hydrolysis of ethyl ester **3c** afforded 3-(benzyl 2-acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-α-D-glucopyranosid-3-yl)propanoic acid (**5**). Coupling of acid **5** with L-2-aminobutanoyl-D-isoglutamine benzyl ester trifluoroacetate and subsequent deprotection of the intermediate **6** furnished *N*-[3-(2-acetamido-2,3-dideoxy-α-D-glucopyranosid-3-yl)propanoyl]-L-2-aminobutanoyl-D-isoglutamine (**7**).

Key words: Carbohydrates; Aminosugars; Muramyl glycopeptides; C–C bond formation; Radical additions; Immunotherapeutics; Peptidoglycan analogs.

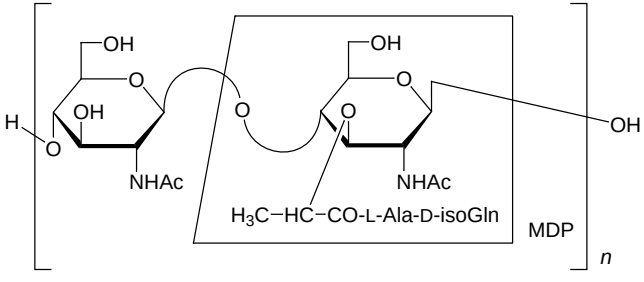
Muramyl glycopeptides, *i.e.*, analogs of "muramyldipeptide" (MDP; MurNAc-L-Ala-D-isoGln) and "glucosaminylmuramyldipeptide" (GMDP; β-D-GlcNAc-(1→4)-MurNAc-L-Ala-D-isoGln) (Fig. 1), are an important group of compounds which are intensively studied as potential immunotherapeutics. (Normuramic acid is the trivial name for 2-amino-3-*O*-(carboxymethyl)-2-deoxy-D-glucopyranose. The symbols and abbreviations obey the published recommendations (International Union of Biochemistry and Molecular Biology; Biochemical Nomenclature and Related Documents, 2nd ed. Portland Press, London 1992).) Some of these compounds are in ad-

+ For preliminary communication see ref.[1]

vanced stage of development as potential immunotherapeutics, *e.g.* "muramyltripeptide" [*N*-(*N*-acetylmuramoyl-L-alanyl-D-isoglutaminyl-L-alanyl)-2-aminoethyl 2(*R*,*S*)-3-di-*O*-palmitoylglyceryl phosphate] and "Romurtid" (MurNAc-L-Ala-D-isoGln-L-Lys(stearoyl)); for a review, see refs[2,3]. One of the factors limiting the use of muramyl glycopeptides in practice is their comparatively low stability under mildly alkaline conditions. These compounds are prone to β-elimination splitting-off of the lactoylpeptide side chain from the reducing end of the saccharide part of their molecule[4]. This problem can be solved, in general, by replacement of the ether bond linking the side chain to C-3 of the saccharide unit with a more stable C–C bond.

RESULTS AND DISCUSSION

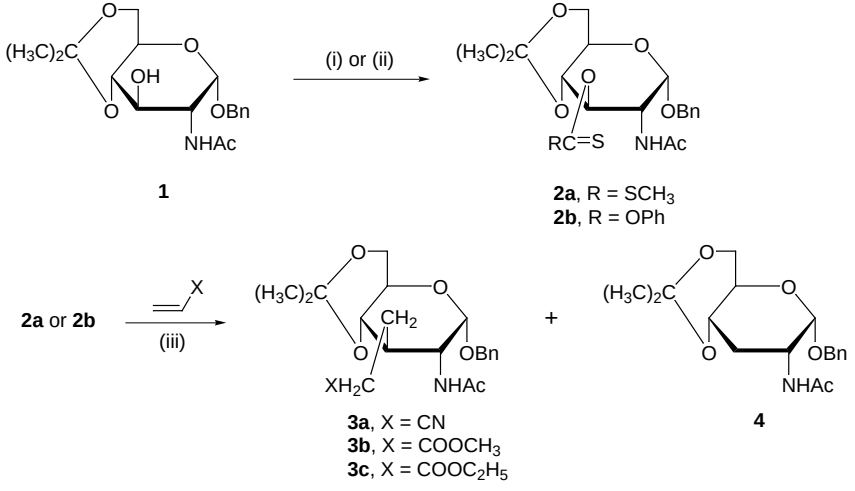
For the synthesis of the key normuramic acid carba analog, we used the tin hydride method (refs[5,6]) to form the C–C bond between the saccharide moiety and the side chain. This reaction is based on the addition of the radical, generated from suitable donor with tributyltin hydride (Bu_3SnH), to an electron-deficient alkene. With regard to the possibility of undesired side reactions, *i.e.*, reduction of radical donors[7] and polymerization of olefins, we studied the influence of the structure of radical donor and radical acceptor on the population of products under different reaction conditions. As radical donors, benzyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[(methylsulfanyl)thiocarbonyl]-α-D-glucopyranoside (**2a**) and benzyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-(phenoxythiocarbonyl)-α-D-glucopyranoside (**2b**) were used, and derivatives of acrylic acid (methyl or ethyl



Peptidoglycan: n = 10–100; GMDP: n = 1

FIG. 1
Structure of peptidoglycan and its fragments

ester and nitrile) were used as electron-deficient olefins (Scheme 1). Reactions were done under different conditions: (A) 2,2′-azobis(isobutyronitrile) (AIBN) in toluene was added to a boiling solution of radical donor **2**, olefin and Bu_3SnH in toluene, and the mixture was refluxed; (B) solution of radical donor **2**, olefin and Bu_3SnH in toluene was refluxed; (C) solution of radical donor **2** and olefin in toluene was gradually added to a boiling solution of Bu_3SnH in toluene. The dithiocarbonate **2a** was prepared by base-catalyzed reaction of benzyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside (**1**, ref.[8]) with carbon disulfide followed by *S*-methylation of the product with CH_3I. The thiocarbonate **2b** was prepared by reaction of compound **1** with phenyl chlorothioformate in the presence of pyridine. The products of addition of radicals, generated from suitable donors **2a** or **2b** with Bu_3SnH, to acrylic acid derivatives are summarized in Table I.



(i) CS_2 in 5M NaOH at r.t. and then CH_3I at 0 °C; (ii) $C_6H_5OC(S)Cl$ and DMAP in dry CH_2Cl_2 and Py at r.t.; (iii) Bu_3SnH in toluene (for details see Table I)

SCHEME 1

Maximum yield of the desired product of addition to the C=C bond, ethyl 3-(benzyl 2-acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-α-D-glucopyranosid-3-yl)propanoate (**3c**) and suppression of unwanted deoxygenation of radical donor **2** to benzyl 2-acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-α-D-*ribo*-hexopyranoside (**4**) were obtained with dithiocarbonate **2a** as radical donor and ethyl acrylate as radical acceptor under the reaction conditions C. The thiocarbonate **2b** was less reactive

than the dithiocarbonate **2a**. Its reaction with ethyl acrylate under conditions C gave a significantly lower yield of the addition product **3c**. Reactions with methyl acrylate led to a higher proportion of deoxy saccharide **4** and a low yield of the desired addition product, methyl 3-(benzyl 2-acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-α-D-glucopyranosid-3-yl)-propanoate (**3b**). This was caused by a higher tendency of methyl acrylate to polymerize. Initiation of this reaction with AIBN (conditions A) led to a significantly lower yield of addition products **3a**–**3c** and enhanced side reactions, *i.e.*, polymerization and deoxygenation. By gradual addition of the acrylate to the reaction mixture under the reaction conditions C, its polymerization could be suppressed and, therefore, the reaction led to a higher proportion of addition products **3a**–**3c** and a lower proportion of deoxy saccharide **4**.

The radical addition proceeded stereoselectively and gave exclusively the product with the *gluco* configuration. Formation of compound with the *allo* configuration was not observed. This high stereoselectivity is apparently caused by the steric effect of protecting groups bonded to the other C-atoms: the C-3 carbon is strongly hindered. Configuration of products

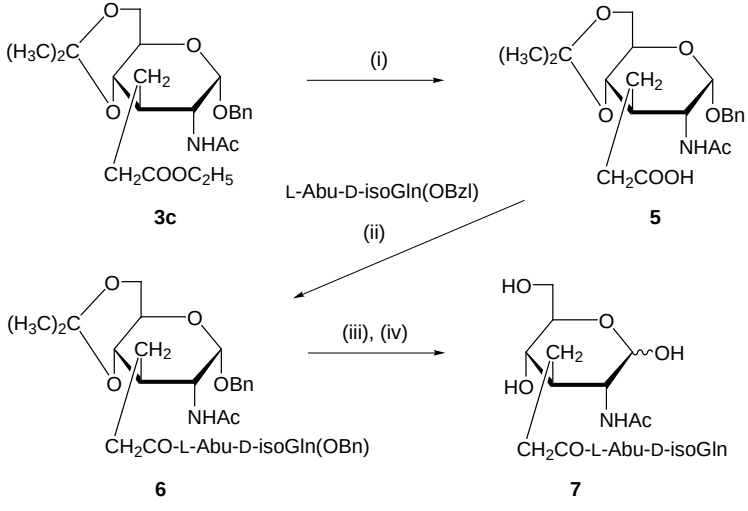
TABLE I
Addition of radical, generated from donors **2a** or **2b** with Bu_3SnH, to the acrylic acid derivatives

Radical donor	CH_2=CH–X	Conditions[a]	Product (yield, %)	
			3	**4**
2a	X = CN	A	**3a** (32)	**4** (47)
2a	X = CN	B	**3a** (35)	**4** (45)
2a	X = CN	C	**3a** (43)	**4** (36)
2a	X = $COOCH_3$	A	**3b** (19)	**4** (57)
2a	X = $COOCH_3$	B	**3b** (28)	**4** (49)
	X = $COOCH_3$	C	**3b** (33)	**4** (46)
2a	X = $COOC_2H_5$	A	**3c** (23)	**4** (55)
	X = $COOC_2H_5$	B	**3c** (50)	**4** (28)
	X = $COOC_2H_5$	C	**3c** (56)	**4** (25)
2b	X = $COOC_2H_5$	C	**3c** (16)	**4** (69)

[a] For the conditions see Results and Discussion.

was determined by ^{1}H NMR using vicinal coupling constants between protons at C-2 and C-3; their high value, 11.5 Hz, corresponds to the *gluco* configuration.

For the preparation of the target muramyl glycopeptide carba analog **7** (Scheme 2), the ethyl ester **3c** was used. It was hydrolyzed with a solution of sodium hydroxide in a mixture of methanol and water, to give 3-(benzyl 2-acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-α-D-glucopyranosid-3-yl)propanoic acid (**5**). Coupling of acid **5** with L-2-aminobutanoyl-D-isoglutamine benzyl ester trifluoroacetate[9] with *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) afforded *N*-[3-(benzyl 2-acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-α-D-glucopyranosid-3-yl)propanoyl]-L-2-aminobutanoyl-D-isoglutamine benzyl ester (**6**). The isopropylidene protecting group was split off from the protected glycopeptide **6** by heating with 80% aqueous acetic acid at 50 °C and the benzyl groups were subse-



(i) 1M NaOH in MeOH at r.t.; (ii) DCC and HOBT in CH_2Cl_2; (iii) 80% CH_3COOH at 50 °C; (iv) H_2 and Pd/C in CH_3COOH at r.t.

SCHEME 2

quently removed by hydrogenolysis on a Pd/C catalyst in acetic acid, to give *N*-[3-(2-acetamido-2,3-dideoxy-α-D-glucopyranosid-3-yl)propanoyl]-L-2-aminobutanoyl-D-isoglutamine (**7**).

In general, this work opened an efficient way to the synthesis of additional carba analogs of muramyl glycopeptides resistant to β-elimination

splitting of the side chain. This fact is of cosiderable importance for practical use of this group of compounds as imunotherapeutics.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Specific rotations were measured on a Perkin–Elmer 141 polarimeter at 22 °C, $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. The IR spectra were recorded on a Bruker IFS 88 (FTIR) spectrometer, wavenumbers are given in cm^{-1}. NMR spectra were recorded with a Varian UNITY-500 spectrometer in the FT mode at 499.8 MHz (^1H) and at 125.6 MHz (^{13}C) in $(CD_3)_2SO$, using the central line of the solvent for standardization (δ 2.50 for ^1H and δ 39.5 for ^{13}C, respectively). Chemical shifts are given in ppm (δ-scale) and coupling constants (*J*) in Hz. Positive-ion FAB mass spectra were measured on a BEqG geometry mass spectrometer ZAB-EQ (VG Analytical, Manchester, U.K.) using an M-Scan FAB gun (Xe, energy 8 keV) at an accelerating voltage of 8 kV. Samples were dissolved in chloroform or methanol and a mixture of glycerol and thioglycerol was used as matrix. Thin-layer chromatography (TLC) was performed on Silufol UV_{254} sheets and column chromatography on silica gel Silpearl (both Kavalier, Votice, Czech Republic). Preparative chromatography was performed on a column filled with Fluka Silica gel 60 (Fluka, Neu-Ulm, Switzerland). Analytical RP HPLC was performed with a Waters apparatus (PDA detector, software Milennium 32; Milford (MA), U.S.A.) equipped with a column (150 × 3.9 mm) of Nova-Pak C18, particle size 4 μm. Preparative RP HPLC was performed on a column (250 × 25 mm) filled with LiChrosorb RP-18, particle size 5 μm (Merck, Darmstadt, Germany). Solutions were evaporated on a rotatory vacuum evaporator. Analytical samples were dried at 6.5 Pa and 25 °C for 8 h.

Benzyl 2-Acetamido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[(methylsulfanyl)thiocarbonyl]-α-D-glucopyranoside (**2a**)

To a stirred solution of benzyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside[8] (**1**; 11.2 g, 32 mmol) in carbon disulfide (9.8 ml) at room temperature, 5 M solution of NaOH (9.8 ml, 49 mmol) and carbon disulfide (6.8 ml, 145 mmol) were added. After dissolving, the mixture was cooled to 0 °C and iodomethane (6.8 ml, 109 mmol) was added under stirring; the stirring was continued at the same temperature for 35 min. The mixture was diluted with ice water (200 ml) and extracted with toluene (3 × 400 ml). Combined organic fractions were dried over anhydrous magnesium sulfate and the solvents were evaporated. Chromatography of the residue on a silica gel column (200 g) in toluene–ethyl acetate (5 : 1) followed by lyophilization from benzene gave 6.48 g (46%) of **2a**; m.p. 53–58 °C, $[\alpha]_D$ +52 (*c* 0.2, chloroform). ^1H NMR: 7.41–7.31 m, 5 H (arom., Bn); 6.16 dd, 1 H, *J* = 9.3, 10.5 (H-3); 5.81 d, 1 H, *J* = 9.8 (NH); 4.90 d, 1 H, *J* = 3.9 (H-1); 4.74 d, 1 H, *J* = 12.0 (CH_2-Ph); 4.50 d, 1 H, *J* = 12.0 (CH_2-Ph); 4.46 ddd, 1 H, *J* = 3.9, 9.8, 10.5 (H-2); 3.96 ddd, 1 H, *J* = 1.5, 8.9, 9.3 (H-4); 3.87–3.76 m, 3 H (H-5,6); 2.54 s, 3 H (SCH_3); 1.87 s, 3 H (CH_3CO); 1.49 s, 3 H (CH_3); 1.38 s, 3 H (CH_3). ^{13}C NMR: 217.7 (C=S); 169.9 (CH_3CO); 136.7–128.1 (arom., Bn); 99.9 [$(CH_3)_2C$]; 97.4 (C-1); 78.9 (C-3); 72.2 (C-5); 69.9 (CH_2-Ph); 64.2 (C-4); 62.3 (C-6); 52.9 (C-2); 29.0 (SCH_3); 23.2 (CH_3CO); 19.0 (CH_3); 18.8 (CH_3). For $C_{20}H_{27}NO_6S_2$ calculated: relative molecular mass 441.6, monoisotopic mass 441.1. FAB MS, *m/z*: 442.2 $[M + H]^+$. For $C_{20}H_{27}NO_6S_2$ (441.5) calculated: 54.40% C, 6.16% H, 3.17% N, 14.52% S; found: 54.69% C, 6.21% H, 2.98% N, 14.28% S.

Benzyl 2-Acetamido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-(phenoxythiocarbonyl)-α-D-glucopyranoside (**2b**)

To a stirred solution of benzyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside[8] (**1**; 1.76 g, 5 mmol) and 4-dimethylaminopyridine (122 mg, 1 mmol) in dry dichloromethane (20 ml) in an apparatus equipped with a septum, pyridine (3 ml) and phenyl chlorothioformate (830 μl, 6 mmol) were added through the septum and the mixture was stirred at room temperature for 8 h. The mixture was diluted with chloroform (100 ml) and extracted with water (3 × 30 ml). The organic layer was dried over anhydrous magnesium sulfate and evaporated. The crude product was chromatographed on a silica gel column (300 g) in toluene–ethyl acetate (3 : 1) to give a syrupy residue of **2b**, which was crystallized from toluene–petroleum ether. Yield 2.0 g (82%) of compound **2b**; m.p. 158–162 °C, $[\alpha]_D$ +31 (*c* 0.4, chloroform). ^{1}H NMR: 7.44–7.06 m, 10 H (arom.); 5.79 dd, 1 H, *J* = 9.1, 10.5 (H-3); 4.91 d, 1 H, *J* = 3.9 (H-1); 4.74 d, 1 H, *J* = 11.9 (CH_2-Ph); 4.51 d, 1 H, *J* = 11.9 (CH_2-Ph); 4.48 ddd, 1 H, *J* = 3.9, 9.5, 10.5 (H-2); 3.92 t, 1 H, *J* = 9.2 (H-4); 3.87-3.76 m, 3 H (H-5,6); 1.92 s, 3 H (CH_3CO); 1.50 s, 3 H (CH_3); 1.43 s, 3 H (CH_3). ^{13}C NMR: 195.9 (C=S); 169.9 (CH_3CO); 153.3, 136.6, 129.4 (2 C); 128.6 (2 C); 128.3, 128.1 (2 C); 126.6 (2 C); 121.70 (arom.); 100.0 [$(CH_3)_2C$]; 97.2 (C-1); 80.6 (C-3); 71.8 (C-5); 69.9 (CH_2-Ph); 63.9 (C-4); 62.2 (C-6); 52.8 (C-2); 23.3 (CH_3CO); 23.3 (CH_3); 18.9 (CH_3). For $C_{25}H_{29}NO_7S$ calculated: relative molecular mass 487.6, monoisotopic mass 487.2. FAB MS, *m/z*: 488.3 $[M + H]^+$. For $C_{25}H_{29}NO_7S$ (487.6) calculated: 61.59% C, 6.00% H, 2.87% N, 6.58% S; found: 61.35% C, 6.18% H, 2.77% N, 6.66% S.

Addition of Free Radical Generated from Thiocarbonates **2a** and **2b** with Tributyltin Hydride to Acrylic Acid Derivatives

Method A: To a stirred boiling solution of compound **2a** (883 mg, 2 mmol), the appropriate derivative of acrylic acid (nitrile, methyl or ethyl ester; 20 mmol) and tributyltin hydride (1.08 ml, 4 mmol) in toluene (20 ml) were added under argon in an apparatus equipped with a septum, followed by 0.25 M solution of AIBN in toluene (4 ml, 1 mmol). The mixture was refluxed for 3 h. The reaction was monitored by TLC in toluene–ethyl acetate (1 : 1). The mixture was evaporated *in vacuo*, the residue was chromatographed on a silica gel column (50 g) in toluene–ethyl acetate (1 : 1) and the products were lyophilized from benzene. The results are summarized in Table I.

Method B: A solution of compound **2a** (883 mg, 2 mmol), the appropriate derivative of acrylic acid (nitrile, methyl or ethyl ester; 20 mmol) and tributyltin hydride (1.08 ml, 4 mmol) in toluene (20 ml) was refluxed under argon for 14 h. The reaction mixture was worked up as described in method *A*. The results are summarized in Table I.

Method C: To a stirred boiling solution of tributyltin hydride (1.08 ml, 4 mmol) in toluene (12 ml) under argon in an apparatus equipped with a septum, a solution of compound **2a** (883 mg, 2 mmol) or **2b** (975 mg, 2 mmol) and the appropriate derivative of acrylic acid (nitrile, methyl or ethyl ester; 20 mmol) in toluene (8 ml) were slowly added through septum during 2 h and the mixture was refluxed for 8 h. The reaction mixture was worked up as described in method *A*. The results are summarized in Table I.

3-(Benzyl 2-acetamido-2,3-dideoxy-4,6-O-isopropylidene-α-D-glucopyranosid-3-yl)propanenitrile (**3a**). Lyophilizate, $[\alpha]_D$ +90 (*c* 0.2, chloroform). IR: 3 436, 3 320 (NH); 3 090, 3 064, 3 032, 1 506, 1 454, 1 023 (Bzl); 2 250 (CN); 1 678 (amide I); 1 540 (amide II); 1 372 (CH_3); 1 134 (tetrahydropyran); 1 124, 1 059 (COC). ^{1}H NMR: 7.41–7.31 m, 5 H (arom.); 5.67 bd, 1 H, *J* = 9.9 (NH); 4.73 d, 1 H, *J* = 11.8 (CH_2-Ph); 4.71 d, 1 H, *J* = 3.6 (H-1); 4.47 d, 1 H, *J* = 11.8

(CH_2-Ph); 4.06 ddd, 1 H, *J* = 3.6, 9.9, 11.5 (H-2); 3.81–3.69 m, 3 H (H-5,6); 3.47 dd, 1 H, *J* = 9.0, 10.5 (H-4); 2.55 ddd, 1 H, *J* = 5.1, 8.0, 16.7 (CH_2CN); 2.44 dt, 1 H, *J* = 8.0, 8.0, 16.7 (CH_2CN); 1.96 s, 3 H (CH_3CO); 1.95 dddd, 1 H, *J* = 2.8, 7.3, 10.5, 11.5 (H-3); 1.88 ddt, 1 H, *J* = 2.8, 8.0, 8.0, 14.9 (CHC**H**$_2$); 1.63 ddt, 1 H, *J* = 5.1, 7.3, 7.9, 14.8 (CHC**H**$_2$); 1.50 s, 3 H (CH_3); 1.40 s, 3 H (CH_3). ^{13}C NMR: 170.1 (CH_3CO); 136.8, 128.7 (2 C); 128.3, 128.2 (2 C) (arom.); 120.2 (CN); 99.5 [$(CH_3)_2$C]; 96.2 (C-1); 73.6 (C-5); 69.7 (CH_2-Ph); 64.9 (C-4); 62.5 (C-6); 50.3 (C-2); 39.7 (C-3); 29.6 (C-7); 29.1 (CH_3); 23.2 (CH_3CO); 19.1 (CH_3); 15.6 (CH_2CN). For $C_{21}H_{28}N_2O_5$ calculated: relative molecular mass 388.5, monoisotopic mass 388.2. FAB MS, *m/z*: 389.1 $[M + H]^+$. For $C_{21}H_{28}N_2O_5$ (388.5) calculated: 64.93% C, 7.27% H, 7.21% N; found: 64.75% C, 6.98% H, 6.95% N.

Methyl 3-(benzyl 2-acetamido-2,3-dideoxy-4,6-O-isopropylidene-α-D-glucopyranosid-3-yl)propanoate (**3b**). Lyophilizate, $[\alpha]_D$ +106 (*c* 0.3, chloroform) .^{1}H NMR: 7.40–7.29 m, 5 H (arom.); 5.82 bd, 1 H, *J* = 9.5 (NH); 4.78 d, 1 H, *J* = 3.7 (H-1); 4.72 d, 1 H, *J* = 11.8 (CH_2-Ph); 4.46 d, 1 H, *J* = 11.8 (CH_2-Ph); 4.03 ddd, 1 H, *J* = 3.7, 9.5, 11.7 (H-2); 3.81–3.64 m, 3 H (H-5,6); 3.65 s, 3 H (CH_3); 3.45 dd, 1 H, *J* = 9.5, 10.2 (H-4); 2.51 ddd, 1 H, *J* = 5.3, 8.4, 16.9 (CH_2COO); 2.39 dt, 1 H, *J* = 8.0, 8.0, 16.9 (CH_2COO); 1.96 s, 3 H (CH_3CO); 1.87 m, 2 H (C**H**$_2$CH, H-3); 1.52 m, 1 H (C**H**$_2$CH); 1.48 s, 3 H (CH_3); 1.39 s, 3 H (CH_3). ^{13}C NMR: 174.4 ($COOCH_3$); 170.0 ($NHCOCH_3$); 137.3, 128.6 (2 C); 128.1 (3 C) (arom.); 99.3 [C$(CH_3)_2$]; 96.5 (C-1); 73.7 (C-5); 69.6 (**C**H_2-Ph); 65.0 (C-4); 62.8 (C-6); 59.8 ($COOCH_3$); 51.0 (C-2); 39.1 (C-3); 31.2 (CH_2COOCH_3); 29.1 (CH_3); 23.2 ($NHCOCH_3$); 23.1 (CH_2CH); 19.0 (CH_3). For $C_{22}H_{31}NO_7$ calculated: relative molecular mass 421.5, monoisotopic mass 421.2. FAB MS, *m/z*: 442.2 $[M + H]^+$. For $C_{22}H_{31}NO_7$ (421.5) calculated: 62.69% C, 7.41% H, 3.32% N; found: 62.47% C, 7.28% H, 3.38% N.

Ethyl 3-(benzyl 2-acetamido-2,3-dideoxy-4,6-O-isopropylidene-α-D-glucopyranosid-3-yl)propanoate (**3c**). Lyophilizate, $[\alpha]_D$ +89 (*c* 0.2, chloroform). ^{1}H NMR: 7.38–7.29 m, 5 H (arom.); 5.84 bd, 1 H, *J* = 9.5 (NH); 4.78 d, 1 H, *J* = 3.6 (H-1); 4.72 d, 1 H, *J* = 11.8 (CH_2-Ph); 4.46 d, 1 H, *J* = 11.8 (CH_2-Ph); 4.11 q, 2 H, *J* = 7.1 (C**H**$_2$CH$_3$); 4.03 ddd, 1 H, *J* = 3.6, 9.5, 11.5 (H-2); 3.84–3.76 m, 3 H (H-5,6); 3.44 dd, 1 H, *J* = 9.5, 10.0 (H-4); 2.48 ddd, 1 H, *J* = 5.4, 8.3, 16.8 (CH_2COO); 2.38 dt, 1 H, *J* = 8.0, 8.0, 16.8 (CH_2COO); 1.96 s, 3 H (CH_3CO); 1.91 dddd, 1 H, *J* = 3.2, 6.8, 10.0, 11.5 (H-3); 1.85 dddd, 1 H, *J* = 3.2, 8.0, 8.3, 14.2 (C**H**$_2$CH); 1.62 s, 3 H (CH_3); 1.55 dddd, 1 H, *J* = 5.4, 6.8, 8.0, 14.2 (C**H**$_2$CH); 1.49 s, 3 H (CH_3); 1.24 t, 3 H, *J* = 7.1 (CH_2C**H**$_3$). ^{13}C NMR: 174.0 ($CH_2COOCH_2CH_3$); 170.0 ($NHCOCH_3$); 137.3, 128.6 (2 C); 128.1(2 C); 128.0 (arom.); 99.4 [$(CH_3)_2$C]; 96.5 (C-1); 73.8 (C-5); 69.6 (CH_2-Ph); 65.1 (C-4); 62.8 (C-6); 60.3 ($COOCH_2CH_3$); 51.1 (C-2); 39.1 (C-3); 31.6 (CH_2CH_2); 29.2 (CH_3); 23.2 ($NHCOCH_3$); 23.1 (CH_2CH_2); 19.1 (CH_3); 14.2 ($COOCH_2CH_3$). For $C_{23}H_{33}NO_7$ calculated: relative molecular mass 435.5, monoisotopic mass 435.2. FAB MS, *m/z*: 436.2 $[M + H]^+$. For $C_{23}H_{33}NO_7$ (435.5) calculated: 63.43% C, 7.64% H, 3.22% N; found: 63.11% C, 7.77% H, 2.99% N.

Benzyl 2-acetamido-2,3-dideoxy-4,6-O-isopropylidene-α-D-ribo-hexopyranoside (**4**). Lyophilizate, $[\alpha]_D$ +42 (*c* 0.3, chloroform). ^{1}H NMR: 7.42–7.30 m, 5 H (arom.); 5.66 bd, 1 H, *J* = 9.8 (NH); 4.77 d, 1 H, *J* = 3.7 (H-1); 4.75 d, 1 H, *J* = 11.6 (CH_2-Ph); 4.47 d, 1 H, *J* = 11.7 (CH_2-Ph); 4.28 dddd, 1 H, *J* = 3.7, 4.8, 9.5, 12.7 (H-2); 3.82 dd, 1 H, *J* = 5.4, 10.5 (H-6); 3.73 t, 1 H, *J* = 10.5, 10.5 (H-6); 3.71 ddd, 1 H, *J* = 4.0, 9.5, 11.2 (H-4); 3.63 ddd, 1 H, *J* = 5.4, 9.5, 10.5 (H-5); 2.01 dt, 1 H, *J* = 4.4, 4.4, 11.1 (CHC**H**$_2$); 1.93 s, 3 H ($COCH_3$); 1.70 dt, 1 H, *J* = 11.2, 11.2, 12.5 (CHC**H**$_2$); 1.50 s, 3 H (CH_3); 1.41 s, 3 H (CH_3). ^{13}C NMR: 169.3 ($COCH_3$); 137.2, 128.6 (2 C); 128.2, 128.1 (2 C) (arom.); 99.4 [$(CH_3)_2$C]; 96.2 (C-1); 69.5 (CH_2-Ph); 68.8 (C-5); 65.5 (C-4); 62.8 (C-6); 47.4 (C-2); 31.2 (C-3); 29.2 (CH_3); 23.3 ($COCH_3$); 19.1 (CH_3). For $C_{18}H_{25}NO_5$

calculated: relative molecular mass 335.4, monoisotopic mass 335.2. FAB MS, *m/z*: 336.2 $[M + H]^+$. For $C_{18}H_{25}NO_5$ (335.4) calculated: 64.46% C, 7.51% H, 4.18% N; found: 64.27% C, 7.73% H, 4.27% N.

3-(Benzyl 2-Acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-α-D-glucopyranosid-3-yl)-propanoic Acid (**5**)

To a stirred solution of ester **3c** (871 mg, 2.0 mmol) in methanol (15 ml), 1 M solution of NaOH (5 ml, 5 mmol) was added and after 4 h at ambient temperature, the mixture was neutralized with Dowex 50W (in the pyridinium form). The ion exchanger was filtered off, washed with methanol (3 × 20 ml) and the filtrate was concentrated *in vacuo*. The residue was codistilled with benzene (3 × 20 ml) and lyophilized from benzene to give 802 mg (98%) of **5**; $[\alpha]_D$ +129 (*c* 0.4, methanol). For $C_{21}H_{29}NO_7$ calculated: relative molecular mass 407.5, monoisotopic mass 407.2. FAB MS, *m/z*: 408.1 $[M + H]^+$, 430 $[M + Na]^+$. For $C_{21}H_{29}NO_7$ (407.5) calculated: 61.90% C, 7.17% H, 3.44% N; found: 62.15% C, 7.05% H, 3.52% N.

N-[3-(Benzyl 2-Acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-α-D-glucopyranosid-3-yl)-propanoyl]-L-2-aminobutanoyl-D-isoglutamine Benzyl Ester (**6**)

A solution of *tert*-butoxycarbonyl-L-2-aminobutanoyl-D-isoglutamine benzyl ester[9] (467 mg, 1.1 mmol) in dichloromethane–trifluoroacetic acid (1 : 1; 20 ml) was kept at room temperature for 30 min and then the mixture was evaporated *in vacuo*. The residue was codistilled with dichloromethane (3 × 15 ml) and dried at room temperature and 1.32 Pa for 2 h. The obtained syrup was dissolved in DMF (5 ml) and the resulting solution of L-2-aminobutanoyl-D-isoglutamine benzyl ester trifluoroacetate was used immediately for coupling with the acid **5**.

To a stirred solution of acid **5** (407 mg, 1.0 mmol) and 1-hydroxybenzotriazole monohydrate (168 mg, 1.1 mmol) in dichloromethane–DMF (9 : 1; 9 ml) at 0 °C, 1 M solution of *N*,*N*′-dicyclohexylcarbodiimide in dichloromethane (1.1 ml, 1.1 mmol) was added and the stirring was continued at 0 °C for 1 h. The precipitated *N*,*N*′-dicyclohexylurea was filtered off (180 mg; 73%), the filtrate was cooled to 0 °C and the above mentioned solution of L-2-aminobutanoyl-D-isoglutamine benzyl ester trifluoroacetate and ethyl(diisopropyl)-amine (366 μl, 2.14 mmol) were added. The mixture was stirred at 0 °C for 1 h and kept overnight at room temperature. Then chloroform (100 ml) was added and the mixture was extracted with saturated solution of sodium hydrogencarbonate (2 × 20 ml) and 5% solution of sodium chloride (3 × 20 ml). The organic phase was dried over anhydrous magnesium sulfate and the solvents were evaporated *in vacuo*. Chromatography of the residue on a silica gel column C18 in solvent system water–methanol (linear gradient 50→100%/60 min) afforded 355 mg (50%) of solid compound **6**; $[\alpha]_D$ +60 (*c* 0.2, chloroform). ^{1}H NMR: 7.30–7.39 m, 10 H (arom.); 7.45 bd, 1 H, *J* = 8.7 (iGln-NH); 7.12 bd, 1 H, *J* = 9.4 (NHAc); 6.76 bd, 1 H, *J* = 7.2 (Abu-NH); 5.14 d, 1 H, *J* = 12.3 (CH_2-Ph); 5.11 d, 1 H, *J* = 12.3 (CH_2-Ph); 4.78 d, 1 H, *J* = 3.9 (H-1); 4.70 d, 1 H, *J* = 11.7 (CH_2-Ph); 4.47 d, 1 H, *J* = 11.7 (CH_2-Ph); 4.34 dt, 1 H, *J* = 5.7, 8.6, 8.6 (α-iGln); 4.12 bq, 1 H, *J* = 7.2 (α-Abu); 4.01 ddd, 1 H, *J* = 3.9, 9.5, 11.6 (H-2); 3.82 dd, 1 H, *J* = 4.8, 10.3 (H-6); 3.72 dd, 1 H, *J* = 10.3, 10.3 (H-6); 3.66 ddd, 1 H, *J* = 4.8, 10.2, 10.2 (H-5); 3.53 dd, 1 H, *J* = 9.2, 10.0 (H-4); 2.51 dt, 1 H, *J* = 7.5, 7.5, 16.9 (γ-iGln); 2.46 ddd, 1 H, *J* = 5.9, 5.9, 12.6 (CH_2CO); 2.43 dt, 1 H, *J* = 7.3, 7.3, 16.9 (γ-iGln); 2.31 ddd, 1 H, *J* = 4.2, 6.4,

12.6 (CH_2CO); 2.16 ddt, 1 H, *J* = 5.7, 7.4, 7.4, 14.3 (β-iGln); 2.06 m, 1 H (C**H**$_2$$CH_2CO$); 1.99 ddt, 1 H, *J* = 7.5, 7.5, 8.6, 14.3 ((β-iGln); 1.97 s, 3 H (CH_3CO); 1.95 m, 1 H, *J* = 4 × 7.4, 13.8 (β-Abu); 1.80 m, 1 H (H-3); 1.58 m, 1 H, *J* = 4 × 7.4, 13.8 (β-Abu); 1.52 s, 3 H (CH_3); 1.44 s, 3 H (CH_3); 1.40 m, 1 H (C**H**$_2$$CH_2CO$); 0.93 t, 3 H, *J* = 7.4 (γ-Abu). ^{13}C NMR: 174.9 (propanoyl-CO); 173.0 (Abu-CO); 172.3 (iGln-CO); 170.8 (iGln-$CONH_2$); 169.3 ($NHCOCH_3$); 136.6, 135.7, 128.7 (2 C); 128.6 (2 C); 128.4 (2 C); 128.4, 128.3, 128.2 (2 C) (all, arom.); 99.6 [$C(CH_3)_2$]; 96.5 (C-1); 74.4 (C-4); 70.0 (CH_2Ph); 66.6 (CH_2Ph); 64.4 (C-5); 62.8 (C-6); 54.8 (Abu-CH); 51.8 (iGln-CH); 51.0 (C-2); 37.9 (C-3); 33.5 (CH_2CO); 30.5 (iGln-CH_2CO); 29.2 [$C(CH_3)_2$]; 27.1 ($CHCH_2$); 25.6 (iGln-$CHCH_2$); 22.9 ($NHCOCH_3$); 23.2 (Abu-CH_2); 19.2 [$C(CH_3)_2$]; 10.4 (Abu-CH_3). For $C_{37}H_{50}N_4O_{10}$ calculated: relative molecular mass 710.8, monoisotopic mass 710.4. FAB MS, *m/z*: 711.6 $[M + H]^+$, 603.5 $[M - OBn + H]^+$. For $C_{37}H_{50}N_4O_{10}$ (710.8) calculated: 62.52% C, 7.09% H, 7.88% N; found: 62.30% C, 7.18% H, 7.65% N.

N-[3-(2-Acetamido-2,3-dideoxy-α/β-D-glucopyranosid-3-yl)propanoyl]-L-2-aminobutanoyl-D-isoglutamine (**7**)

The fully protected derivative **6** (213 mg, 0.3 mmol) was heated under stirring in 80% aqueous acetic acid (5 ml) at 50 °C for 1 h and the solvents were evaporated *in vacuo*. The product in acetic acid (50 ml) was hydrogenolyzed in the presence of 5% Pd/C (200 mg) at room temperature for 24 h. The apparatus was purged with argon and the catalyst was filtered off and washed with acetic acid (50 ml). The filtrate was lyophilized and the product was chromatographed on a silica gel C18 column in methanol–water (1 : 19). The homogeneous fractions corresponding to α/β-anomeric mixture of **7** were evaporated *in vacuo* and the residue was lyophilized from water. Yield 109 mg (74%) of compound **7**; $[\alpha]_D$ +14 (*c* 0.2, water). ^{1}H NMR (α/β 1 : 1); α-glucopyranoside: 5.11 d, 1 H, *J* = 3.5 (H-1); 3.90 dd, 1 H, *J* = 2.3, 12.2 (H-6); 3.81 dd, 1 H, *J* = 3.5, 11.0 (H-2); 3.73 dd, 1 H, *J* = 5.9, 12.2 (H-6); 3.48 ddd, 1 H, *J* = 2.3, 5.9, 9.5 (H-5); 3.42 t, 1 H, *J* = 9.6, 9.6 (H-4); 2.37–2.40 m, 2 H (CH_2CO); 2.04 s, 3 H ($NHCOCH_3$); 1.95–1.97 m, 3 H (H-3, C**H**$_2$CH); β-glucopyranoside: 4.67 d, 1 H, *J* = 8.2 (H-1); 3.84 dd, 1 H, *J* = 2.0, 12.2 (H-6); 3.82 ddd, 1 H, *J* = 2.0, 5.5, 9.6 (H-5); 3.78 dd, 1 H, *J* = 5.5, 12.2 (H-6); 3.59 dd, 1 H, *J* = 8.2, 11.3 (H-2); 3.46 t, 1 H, *J* = 9.7, 9.7 (H-4); 2.37–2.40 m, 2 H (CH_2CO); 2.04 s, 3 H ($NHCOCH_3$); 1.95–1.97 m, 3 H (H-3, C**H**$_2$CH); dipeptide (the same values for both anomers): 4.35 dd, 1 H, *J* = 4.8, 10.0 (α-iGln); 4.16 dd, 1 H, *J* = 5.2, 6.4 (α-Abu); 2.49 ddd, 1 H, *J* = 6.1, 8.1, 16.9 (γ-iGln); 2.46 ddd, 1 H, *J* = 7.5, 14.7, 16.9 (γ-iGln); 2.17–2.24 m, 1 H (β-iGln); 1.98 dddd, 1 H, *J* = 6.1, 7.5, 10.0, 14.7 (β-iGln); 1.72–1.85 m, 2 H (β-Abu); 0.97 t, 3 H, *J* = 7.4 (γ-Abu). ^{13}C NMR; α-glucopyranoside: 90.2 (C-1); 72.6 (C-5); 67.4 (C-4); 61.2 (C-6); 53.2 (C-2); 39.2 (C-3); β-glucopyranoside: 96.7 (C-1); 79.2 (C-5); 67.5 (C-4); 61.4 (C-6); 56.1 (C-2); 44.5 (C-3); dipeptide (the same values for both anomers): 176.9 (Abu-CO); 176.4 (iGln-CO); 175.2 (iGln-CH_2COOH); 53.4 (Abu-CH); 51.0 (iGln-CH); 30.9 (iGln-CH_2COOH); 26.4 (iGln-$CHCH_2$); 24.7 (Abu-CH_2); 9.8 (Abu-CH_3). For $C_{20}H_{34}N_4O_{10}$ calculated: relative molecular mass 490.5, monoisotopic mass 490.2. FAB MS, *m/z*: 513.2 $[M + Na]^+$, 535.1 $[M + 2\ Na]^+$. For $C_{20}H_{34}N_4O_{10}$ (490.5) calculated: 48.97% C, 6.99% H, 11.42% N; found: 48.78% C, 7.13% H, 11.29% N.

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