NEW EFFECTIVE SYNTHESIS OF (*N*-ACETYL- AND *N*-STEAROYL-2- AMINO-2-DEOXY-β-D-GLUCOPYRANOSYL)-(1→4)-*N*-ACETYLNORMURAMOYL-L-2-AMINOBUTANOYL-D-ISOGLUTAMINE, ANALOGS OF GMDP WITH IMMUNOPOTENTIATING ACTIVITY

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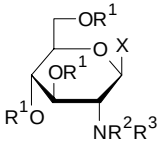
Ethyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**5**), prepared by benzylation of ethyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**4**), was transformed by reaction with bromine into 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**6**). Thioglycoside **5** in the presence of methyl triflate and glycosylbromide **6** in the presence of silver triflate were used as glycosyl donors for condensation with benzyl 2-acetamido-3-*O*-allyl-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**7**), to give benzyl 2-acetamido-3-*O*-allyl-6-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-deoxy-α-D-glucopyranoside (**8**). Its reductive dephthaloylation with $NaBH_4$/AcOH afforded benzyl 2-acetamido-3-*O*-allyl-4-*O*-(2-amino-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**11**). Compound **11** was *N*-acylated to give benzyl 2-acetamido-4-*O*-(2-acylamino-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-3-*O*-allyl-6-*O*-benzyl-2-deoxy-α-D-glucopyranosides (**12a**) or (**12b**). These compounds were converted into corresponding benzyl 2-acetamido-4-*O*-(2-acylamino-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-3-*O*-carboxymethyl-2-deoxy-α-D-glucopyranosides which, by condensation with H-L-Abu-D-isoGln(OBzl) followed by hydrogenolysis of protective benzyl groups, furnished glycopeptides **16a** and **16b**. Intramolecular O→N migration of the allyl protecting group followed by its reduction to the propyl residue by reaction of compound **8** with hydrazine or hydrazinium acetate, to give benzyl 2-acetamido-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-propylamino-β-D-glucopyranosyl)-6-*O*-benzyl–2-deoxy-α-D-glucopyranoside (**9**), is also described.

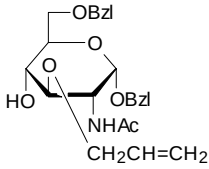
Key words: Carbohydrates; Glycosides; Aminosugars; Oligosaccharides; Muramyl glycopeptides; Immunostimulators.

Some time ago we described the synthesis of (2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-*N*-acetylnormuramoyl-L-2-aminobutanoyl-D-isoglutamine (**16a**, β-D-GlcNAc-(1→4)-norMurNAc-L-Abu-D-isoGln), an analogue of GMDP (β-D-GlcNAc-(1→4)-MurNAc-L-Ala-D-isoGln) modified both in the sugar and peptide parts of the molecule, and its lipophilic derivative (2-deoxy-2-stearamido-β-D-glucopyranosyl)-(1→4)-*N*-ace-

tylnormuramoyl-L-2-aminobutanoyl-D-isoglutamine* (**16b**, β-D-GlcNstearoyl-(1→4)-norMurNAc-L-Abu-D-isoGln), bearing a bulky stearoyl residue on the NH_2 group of glucosamine subunit (refs[1–3]). Both compounds, compared with MDP (muramoyl-dipeptide) and GMDP, exhibit higher immunopotentiating activity and their undesirable side effects are suppressed (*e.g.*, they are not pyrogenic). Immunoadjuvant activity of the lipophilic derivative **16b** is fully comparable with the activity of FCA (Freund's Complet Adjuvant). Compound **16a** and especially its lipophilic derivative **16b** stimu-



1, R^1= Ac, R^2= R^3= H, X = OAc
2, R^1= Ac, R^2+ R^3= Pht, X = OAc
3, R^1= Ac, R^2+ R^3= Pht, X = SEt
4, R^1= H, R^2+ R^3= Pht, X = SEt
5, R^1= Bzl, R^2+ R^3= Pht, X = SEt
6, R^1= Bzl, R^2+ R^3= Pht, X = Br



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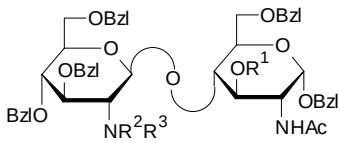
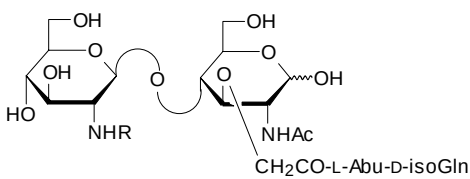


8, R^1= All, R^2= R^3= Pht
9, R^1= H, R^2= H, R^3= $(CH_2)_2CH_3$
10, R^1= R^2= R^3= H
11, R^1= All, R^2= R^3= H
12a, R^1= All, R^2= H, R^3= Ac
12b, R^1= All, R^2= H, R^3= stearoyl

13a, R^1= R^2= H, R^3= Ac
13b, R^1= R^2= H, R^3= stearoyl
14a, R^1= CH_2COOH, R^2= H, R^3= Ac
14b, R^1= CH_2COOH, R^2= H, R^3= stearoyl
15a, R^1= CH_2CO-L-Abu-D-isoGln(OBzl), R^2= H, R^3= Ac
15b, R^1= CH_2CO-L-Abu-D-isoGln(OBzl), R^2= H, R^3= stearoyl



All = $CH_2CH=CH_2$
Bzl = $CH_2C_6H_5$
Pht = phthaloyl

16a, R = Ac
16b, R = stearoyl

* Normuramic acid is the trivial name for 2-amino-3-*O*-carboxymethyl-2-deoxy-D-glucopyranose. The symbols and abbreviations obey the published recommendations (*Biochemical Nomenclature and Related Documents.* International Union of Biochemistry, London 1978).

late haemopoiesis and possess significant protective and therapeutic effects in radiation injury and restore lymphopenia caused by some xenobiotics (for review, see refs[4,5]).

Now we describe a new effective preparation of disaccharide precursors **12a** and **12b** from which compounds **16a** and **16b** can be prepared. For the synthesis of the key disaccharide **8** we chose an approach based on condensation of monosaccharide units with protecting groups representing an orthogonal system. As glycosyl acceptor we used benzyl 2-acetamido-3-*O*-allyl-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**7**, ref.[1]) and as a glycosyl donor, or its precursor, we used ethyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**5**). The use of glycosyl donor protected by *O*-benzyl groups instead of the often used *O*-acetyl groups enabled us to avoid the cumbersome exchange of the protecting groups after the demanding glycosylation step (see, *e.g.*, refs[1,6]). The glycosyl donor **5** was obtained by *O*-benzylation of ethyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**4**, ref.[7]) by benzylbromide in *N*,*N*-dimethylformamide in the presence of sodium hydride.

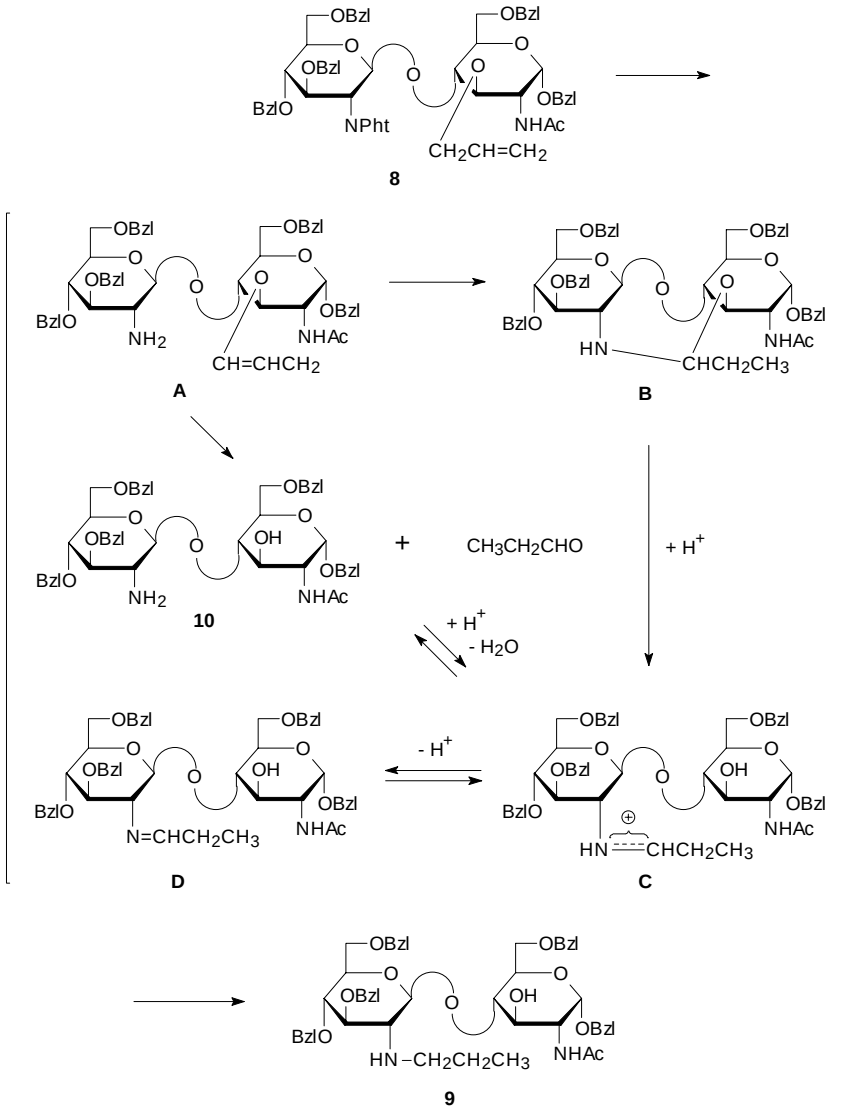
Ethyl thioglycoside **4** was prepared from 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose (**1**) in three steps by a modified procedure[7,8]. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**2**) was obtained by the reaction of amine **1** with phthalic anhydride in dichloromethane in the presence of 4-dimethylaminopyridine (DMAP) and subsequent cyclization of the intermediate phthalamic acid with acetic anhydride. Ethyl chloroformate and triethylamine used in the cyclization step in ref.[7], were replaced by acetic anhydride in the presence of DMAP with the aim to eliminate the undesirable reaction leading to isophthalimide[9,10]. Our procedure, in comparison with described[9,11,12] ones, gave phthalimido derivative **2** in consistently high yield (82%), and is also suitable for its preparation in a larger scale. The reaction of the phthalimido derivative **2** with ethanethiol in dichloromethane promoted by titanium tetrachloride afforded thioglycoside **3**. The *O*-acetyl groups of **3** were removed with sodium methoxide in methanol to give compound **4**.

Methyl triflate-promoted glycosylation[13,14] of glycosyl acceptor **7** with glycosyl donor **5** afforded benzyl 2-acetamido-3-*O*-allyl-6-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-deoxy-α-D-glucopyranoside (**8**) with the yield of only 20%. Owing to the low effectivity of the glycosylation proceeding *via* sulfonium ion, we used an alternative approach. Thioglycoside **5** was transformed with bromine in dichloromethane at 0 °C (ref.[15]) to 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosylbromide (**6**), which by reaction with glycosyl acceptor **7** in the presence of silver triflate (molar ratio 3 : 2 : 3) in dichloromethane at –45 °C afforded the desired disaccharide **8** in satisfactory yield of 73%. This reaction was carried out without base because, in the case of little reactive acceptor[1,2], the base acts as a glycosylation inhibitor.

The attempt to dephthaloylate disaccharide **8** with butylamine in boiling methanol, *i.e.*, under the conditions described[1] for the cleavage of the phthalimido group from

benzyl 2-acetamido-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-3-*O*-allyl-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside, was not successful. This negative result is probably due to the presence of alkali-stable benzyl group on the vicinal 3′-OH group in disaccharide **8**. In the case of the above mentioned disaccharide, which is protected by alkali-labile *O*-acetyl groups, the acetyls are preferentially cleaved by butylamine and the free 3′-OH group can participate in the dephthaloylation step. The strong accelerating effect of vicinal 3′-OH group on base-catalyzed *N*-deacetylation of 2-acetamido-2-deoxy-D-glucopyranosyl residue has been described[3]. Also the attempt to dephthaloylate compound **8** under harsher conditions, *i.e.* by hydrazine in boiling ethanol, was not successful. Under these conditions, the dephthaloylation was followed by the O→N migration and reduction of the allyl protecting group to the propyl residue, and as a product, the benzyl 2-acetamido-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-propylamino-β-D-glucopyranosyl)-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**9**) was only isolated. An analogous result was obtained also by dephthaloylation of compound **8** with hydrazine acetate in boiling ethanol. In the literature, only migration of the allyl group to oxygen, nitrogen, carbon, sulfur and hydride nucleophiles by transition metal complex-catalyzed cleavage is known, and these compounds, as well as hydride donors (*e.g.*, formic acid), are used as allyl scavengers[16,17]. This unexpected result can be explained by transformation resembling to the intramolecular Leuckart–Wallach reaction as shown in Scheme 1. The reaction of compound **8** with hydrazine or hydrazine acetate leads, besides cleavage of the phthalimido group, also to isomerization of the allyl protecting group to propenyl group under formation of **A**. The subsequent intramolecular addition of free NH_2-group to vinyl ether system of the propenyl group affords cyclic intermediate **B**. The action of formic acid added during workup of the reaction mixture opens the cyclic compound **B** to give ion **C**. Reduction of **C** by formic acid affords the *N*-propyl derivative **9**. We consider an alternative intermolecular course of this reaction based on the cleavage of the propenyl group of the intermediate **A** by formic acid to give benzyl 2-acetamido-4-*O*-(2-amino-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**10**) and propionaldehyde and their subsequent Leuckart–Wallach reaction to compound **9** to be less probable, owing to a high yield of propyl derivative **9**. The presence of **10** in the reaction mixture was not observed. Compound **10** was prepared from benzyl 2-acetamido-3-*O*-allyl-4-*O*-(2-amino-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**11**) by cleavage of the allyl protecting group *via* its catalytic isomerization to propenyl group by Wilkinson's catalyst [$(Ph_3P)_3RhCl$] followed by acidic hydrolysis. Amine **11** was prepared by two-steps reductive dephthaloylation[11,18] of compound **8**. The phthalimido group was reduced with $NaBH_4$ to the corresponding 2-(hydroxymethyl)benzamide, which was in the second step cleaved by acetic acid at 85 °C.

N-Acylation of amine **11** with acetic anhydride or stearoyl chloride in a mixture of pyridine and dichloromethane afforded benzyl 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-3-*O*-allyl-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**12a**, ref.[1]) or benzyl 2-acetamido-3-*O*-allyl-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-stearoylamino-β-D-glucopyranosyl)-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**12b**, ref.[3]), respectively. These compounds can be transformed to the target glycopeptides,



SCHEME 1

(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-*N*-acetylnormuramoyl-L-2-aminobutanoyl-D-isoglutamine (**16a**) and (2-deoxy-2-stearamido-β-D-glucopyranosyl)-(1→4)-*N*-acetylnormuramoyl-L-2-aminobutanoyl-D-isoglutamine (**16b**), respectively, in four steps, according to our earlier described procedure[1,3]: (i) cleavage of the allyl protecting group, (ii) *O*-alkylation of the formed hydroxy derivatives **13a** and **13b** by chloroacetic acid in the presence of sodium hydride, (iii) DCC-promoted condensation of the 3-*O*-carboxymethyl derivatives **14a** and **14b** with H-L-Abu-D-isoGln(OBzl) and (iv) hydrogenolysis of benzyl protecting groups from glycopeptides **15a** and **15b**.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured on a Perkin–Elmer 141 polarimeter at 22 °C. 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 deuteriochloroform, using tetramethylsilane as internal standard for the ^1H NMR spectrum and deuteriochloroform (δ 77.0) as standards for ^{13}C NMR spectrum. Chemical shifts are given in ppm (δ-scale) and coupling constants (*J*) in Hz. For uninterchangeable assignment of signals in ^{13}C NMR spectra of compounds **8**, **10**, **11** and **12a**, the heterocorrelated 2D NMR spectra were measured by the HMQC technique using the standard pulse sequence delivered by the producer of the spectrometer. Following set of parameters was used: spectral width in both f_1 and f_2 dimensions 4 500 Hz and 17 000 Hz, respectively, number of scans 32, number of increments in f_1 dimension 256, recycle delay 1 s, acquisition time 0.2 s, 90° pulse for ^1H was 22.5 μs, data matrix for processing 2 048 × 2 048 datapoints, for processing no weighting function was used. 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 the mixture glycerol–thioglycerol or 3-nitrobenzyl alcohol 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). Analytical RP HPLC was performed with a Spectra-Physics 8700 apparatus (Darmstadt, Germany) equipped with a column (250 × 4 mm) filled with Separon SGX-RPS (C18), particle size 10 nm (Tessek, Prague). Preparative RP HPLC was performed with a Knauer apparatus (Bad Homburg, Germany) equipped with a column (250 × 10 mm) filled with Separon SGX-RPS, particle size 10 nm (Tessek, Prague). Solutions were evaporated on rotatory vacuum evaporator. Analytical samples were dried at 6.5 Pa and 25 °C for 8 h.

Dichloromethane was distilled from phosphorus pentoxide and stored over molecular sieves 4A. Silver trifluoromethanesulfonate was recrystallized from toluene.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**2**)

Solution of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose[8] (**1**, 173.7 g, 0.5 mol), phthalic anhydride (75.54 g, 0.51 mol) and 4-dimethylaminopyridine (10.0 g, 81.8 mmol) in dichloromethane (1 500 ml) was stirred at ambient temperature until of the starting amine **1** disappeared; the reaction was monitored by TLC in ethyl acetate–chloroform–formic acid (20 : 10 : 1). Acetic anhydride was added (300 ml, 3.18 mol) and the mixture was allowed to stand overnight; the reaction was monitored by TLC in ethyl acetate–toluene (1 : 1). Under stirring, ethanol (200 ml) was added and, after 2 h at ambient temperature the solvents were evaporated and the residue was codistilled with toluene (3 × 500 ml) to give a syrup, which crystallized on addition of ethanol and standing

overnight at +3 °C, to yield crude **2** (195.4 g, 82%); m.p. 72–75 °C. Recrystallization of the product from ethanol afforded 175.8 g (75%) of compound **2**; m.p. 79–81 °C, $[\alpha]_D$ +62° (*c* 0.56, chloroform); ref.[9]: m.p. 69–71 °C; ref.[11]: m.p. 74–75 °C, $[\alpha]_D$ +71° (*c* 1.3, chloroform). IR spectrum (tetrachloromethane): 3 088, 3 062, 3 028 (C–H, Pht); 1 783, 1 725 (C=O, Pht); 1759 (C=O, Ac); 1 615, 1 592, 1 470, 1 384 (arom. ring). ^{1}H NMR spectrum: 7.86–7.88 m, 7.75–7.77 m, 4 H (H-arom.); 6.52 d, 1 H, *J* = 8.8 (H-1); 5.89 dd, 1 H, *J* = 9.0, 10.5 (H-3); 5.22 dd, 1 H, *J* = 9.0, 10.3 (H-4); 4.47 dd, 1 H, *J* = 8.8, 10.5 (H-2); 4.37 dd, 1 H, *J* = 4.3, 12.4 (H-6a); 4.15 dd, 1 H, *J* = 2.2, 12.4 (H-6b); 4.03 ddd, 1 H, *J* = 2.2, 4.3, 10.3 (H-5); 1.87 s, 2.00 s, 2.05 s, 2.12 s, 4 × 3 H (3 × CH_3CO). ^{13}C NMR spectrum: 170.7 s, 170.1 s, 169.5 s, 168.7 s (4 × CH_3COO); 167.4 s, 2 × C (2 × C=O, Pht); 134.5 d, 2 × C, 131.2 s, 2 × C, 123.8 d, 3 × 2 C (Pht); 89.8 d (C-1); 72.6 d (C-5); 70.5 d (C-3); 68.3 d (C-4); 61.5 t (C-6); 53.5 d (C-2); 20.7 q, 20.7 q, 20.6 q, 20.4 q (4 × CO**C**H_3). For $C_{22}H_{23}NO_{11}$ calculated: relative molecular mass 477.4, monoisotopic mass 477.1. FAB MS, *m/z*: 478.3 $[M + H]^+$, 500.3 $[M + Na]^+$. For $C_{22}H_{23}NO_{11}$ (477.4) calculated: 55.34% C, 4.85% H, 2.93% N; found: 55.19% C, 4.85% H, 2.85% N.

Ethyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**3**)

To a stirred solution of compound **2** (167 g, 0.35 mol) and ethanethiol (66 ml, 0.89 mol) in dry dichloromethane (1 700 ml) in the presence of powdered molecular sieves 4A (150 g) at 0 °C titanium tetrachloride (50 ml, 0.46 mol) was slowly added. After 1 h stirring at ambient temperature the reaction mixture was filtered through a layer of cellite and the cellite was washed with dichloromethane (1 500 ml). Collected filtrate was washed with 1 M H_2SO_4 (800 ml), saturated solution of sodium hydrogen carbonate till neutral pH, and water (800 ml), dried over anhydrous magnesium sulfate, and the solvent was evaporated. The residue was crystallized from a mixture of ethyl acetate and petroleum ether. Yield 121 g (72%) of compound **3**; m.p. 115–118 °C, $[\alpha]_D$ +46° (*c* 0.8, dichloromethane); ref.[7]: m.p. 118–119 °C, $[\alpha]_D$ +44° (*c* 0.8, dichloromethane). For $C_{22}H_{25}NO_9S$ calculated: relative molecular mass 479.5, monoisotopic mass 479.1. FAB MS, *m/z*: 480.1 $[M + H]^+$, 502.1 $[M + Na]^+$. For $C_{22}H_{25}NO_9S$ (479.5) calculated: 55.10% C, 5.25% H, 2.92% N, 6.68% S; found: 54.94% C, 5.11% H, 2.98% N, 6.46%S.

Ethyl 2-Deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**4**)

A suspension of compound **3** (128 g, 0.27 mol; dried 6 h at room temperature and 1.32 Pa) in 0.01 M CH_3ONa in methanol (2 500 ml) was stirred at room temperature for 2 h; during this time the suspension dissolved. The solution was allowed to stand overnight at 3 °C and neutralized by addition of Dowex 50 (pyridinium form). The ion exchanger was filtered off, washed with methanol and the filtrate was concentrated *in vacuo*. Crystallization of the residue from dichloromethane afforded 85.5 g (90%) of product **4**; m.p. 169–170 °C, $[\alpha]_D$ +9° (*c* 0.6, methanol); compound **4** is described in ref.[7] as a non characterized intermediate. IR spectrum (KBr): 3 494, 3 458, 3 361 (O–H, bonded); 3 109, 3 051, 3 026 (C–H, Pht); 1 767, 1 754, 1 748, 1 700, 1 673 (C=O, Pht); 1 611, 1 466, 1 394, 1 383 (arom. ring). For $C_{16}H_{19}NO_6S$ calculated: relative molecular mass 353.4, monoisotopic mass 353.1. FAB MS, *m/z*: 376.0 $[M + Na]^+$. For $C_{16}H_{19}NO_6S$ (353.4) calculated: 54.38% C, 5.41% H, 3.96% N, 9.07% S; found: 54.55% C, 5.24% H, 3.95% N, 9.04% S.

Ethyl 3,4,6-Tri-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**5**)

A solution of compound **4** (42.4 g, 120 mmol) and benzyl bromide (85.6 ml, 720 mmol) in dry *N*,*N*-dimethylformamide (600 ml) was slowly added to a stirred 60% suspension of NaH in mineral oil (28.8 g, 720 mmol) at 0 °C during 30 min under nitrogen, and the mixture was stirred at ambient

temperature overnight. Acetic anhydride (350 ml, 3.7 mol) was added and stirring continued for 3 h. The solution was poured under stirring and ice-bath cooling to a saturated sodium hydrogen carbonate solution (2 l). The product was extracted with toluene (3 × 600 ml), the extracts were dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. Chromatography of the residue on a silica gel column (1 000 g) in toluene–ethyl acetate (20 : 1) afforded 65.5 g (88%) of syrupy product, which was crystallized from toluene–petroleum ether. Yield 54.1 g (72%) of compound **5**; m.p. 103–104 °C, $[\alpha]_D$ +63° (*c* 0.4, chloroform). IR spectrum (tetrachloromethane): 3 089, 3 066, 3 032 (C–H, Bzl, Pht); 1 778, 1 718 (C=O, Pht); 1 612, 1 588, 1 497, 1 470, 1 454, 1 387 (arom. ring Bzl, Pht). ^{1}H NMR spectrum: 6.83–7.80 m, 3 × 5 H (H-arom., Bzl); 6.83–7.50 m, 4 H (H-arom., Pht); 5.26 d, 1 H, *J* = 10.5 (H-1); 4.84 d, 1 H, *J* = 10.5 (CH_2–Ph); 4.79 d, 1H, *J* = 12.0 (CH_2–Ph); 4.65 d, 1H, *J* = 12.0 (CH_2–Ph); 4.65 d, 1 H, *J* = 10.5 (CH_2–Ph); 4.58 d, 1 H, *J* = 12.0 (CH_2–Ph); 4.45 d, *J* = 12.0 (CH_2–Ph); 4.39 dd, 1 H, *J* = 8.7, 10.3 (H-3); 4.26 t, 1 H, *J* = 10.3 (H-2); 3.81 dd, 1 H, *J* = 2.4, 11.0 (H-6b); 3.78 dd, 1 H, *J* = 4.1, 11.0 (H-6a); 3.78 dd, 1 H, *J* = 8.5, 10.0 (H-4); 3.68 ddd, 1 H, *J* = 2.4, 4.1, 10.0 (H-5); 2.69 dq, 1 H, *J* = 7.3, 12.7 (CH_3CH**H**S); 2.60 dq, 1 H, *J* = 7.3, 12.7 (CH_3C**H**HS); 1.18 t, 3 H, *J* = 7.3 (C**H**$_3CH_2$S). ^{13}C NMR spectrum: 168.0 s, 167.5 s, 138.3 s, 138.0 s (2 × C), 133.8 d, 133.7 d, 132.0 s, 131.7 s, 128.9 d (2 × C), 128.4 d (2 × C), 128.1 d (3 × C), 127.9 d (4 × C), 127.8 d, 127.7 d, 127.6 d, 127.3 d, 123.4 d, 123.2 d (arom. C, Bzl, Pht); 81.0 d (C-1); 80.4 d (C-3); 79.6 d (C-4); 79.4 d (C-5); 75.0 t, 74.9 t, 73.4 t (**C**H$_2$–Ph); 68.9 t (C-6); 55.0 d (C-2); 23.86 t (**C**H$_3$CH$_2$S); 14.9 q (CH_3**C**H$_2$S). For $C_{37}H_{37}NO_6S$ calculated: relative molecular mass 623.8, monoisotopic mass 623.2. FAB MS, *m/z*: 624.1 $[M + H]^+$, 646.1 $[M + Na]^+$. For $C_{37}H_{37}NO_6S$ (623.8) calculated: 71.24% C, 5.97% H, 2.24% N, 5.14% S; found: 71.05% C, 5.99% H, 2.18% N, 5.30% S.

Benzyl 2-Acetamido-3-*O*-allyl-6-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-deoxy-α-D-glucopyranoside (**8**)

Method A. A mixture of compound **5** (312 mg, 0.5 mmol), benzyl 2-acetamido-3-*O*-allyl-6-*O*-benzyl-2-deoxy-D-glucopyranoside (**7**, ref.[1]; 221 mg, 0.5 mmol) and molecular sieves 4A (0.8 g) was dried in an apparatus with a septum, for 8 h at room temperature and 1.32 Pa. The apparatus was flushed with argon (2 ×) and dry dichloromethane (10 ml) was added through the septum. After dissolution, the mixture was cooled to –15 °C and methyl triflate (226 μl, 2 mmol) was added under stirring through the septum and the mixture was allowed to stand at –15 °C for 3 days. Triethylamine (200 μl, 1.4 mmol) was added and after 5 min stirring, the mixture was filtered. The filtrate was diluted with chloroform (70 ml) and the solution was washed with 1 M H_2SO_4, saturated $NaHCO_3$ and water (3 × 20 ml each), dried over anhydrous magnesium sulfate, and the solvents were evaporated. Chromatography of the residue on silica gel column (30 g) in toluene–ethyl acetate (2 : 1) followed by lyophilization from benzene afforded 105 mg (21%) of product **8**; m.p. 50–54 °C, $[\alpha]_D$ +63° (*c* 0.5, chloroform). IR spectrum (tetrachloromethane): 3 449 (NH, NHAc); 3 089, 3 066, 3 033 (C–H, Bzl, Pht, All); 1 779, 1 717 (C=O, Pht); 1 692 (amide I); 1 614, 1 587, 1 497, 1 469, 1 454, 1 388 (arom. ring, Bzl, Pht); 1 508 (amide II). ^{1}H NMR spectrum: 6.88–7.80 m, 19 H (arom. H, Bzl, Pht); 5.77 dddd, 1 H, *J* = 4.6, 6.1, 10.5, 17.2 (H-2″); 5.48 bd, 1 H, *J* = 9.3 (N**H**$COCH_3$); 5.29 d, 1 H, *J* = 8.6 (H-1′); 5.17 ddt, 1 H, *J* = 1.7, 1.7, 2.0, 17.2 (H-3a″); 4.99 ddt, 1 H, *J* = 1.4, 1.4, 1.7, 10.5 (H-3b″); 4.82 d, 1 H, *J* = 3.6 (H-1); 4.80 d, 1 H, *J* = 11.2 (CH_2–Ph); 4.78 d, 1 H, *J* = 12.2 (CH_2–Ph); 4.67 d, 1 H, *J* = 11.2 (CH_2–Ph); 4.64 d, 1 H, *J* = 12.2 (CH_2–Ph); 4.54 d, 1 H, *J* = 12.2 (CH_2–Ph); 4.53 d, 1 H, *J* = 11.9 (CH_2–Ph); 4.41 d, 1 H, *J* = 12.2 (CH_2–Ph); 4.39 d, 1 H, *J* = 12.2 (CH_2–Ph); 4.38 ddt, 1 H, *J* = 1.5, 1.5, 4.6, 13.1 (H-1a″); 4.34 d, 1 H, 11.9 (CH_2–Ph); 4.34 d, 1 H, *J* = 12.2 (CH_2–Ph); 4.33 dd, 1 H, *J* = 8.8, 11.0 (H-3′); 4.16 ddd, 1 H, *J* = 3.6, 9.3, 10.5 (H-2); 4.14 dd, 1 H, *J* = 8.6, 11.0 (H-2′); 4.01 dd, 1 H, *J* = 8.8, 9.8 (H-4); 3.99 ddt, 1 H, *J* = 1.5, 1.5, 6.1, 13.1 (H-1b″); 3.86 dd, 1 H, *J* = 8.8, 9.8 (H-4′); 3.75 dd, 1 H, *J* = 3.2, 11.1 (H-6a′); 3.72 dd, 1 H, *J* = 2.2, 11.1

(H-6b′); 3.52 dt, 1 H, *J* = 2.7, 2.7, 9.8 (H-5); 3.50 dd, 1 H, *J* = 8.8, 10.5 (H-3); 3.43 ddd, 1 H, *J* = 2.2, 3.2, 9.8 (H-5′); 3.34–3.38 m, 2 H (H-6a and H-6b); 1.91 s, 3 H (NHCOC**H**$_3$). ^{13}C NMR spectrum: 169.7 s (NH**C**OCH$_3$); 168.0 s, 167.8 s, 138.3 s (2 × C), 138.1 s, 138.0 s, 137.1 s, 133.7 d, 131.8 s, 131.4 s, 128.4 d (4 × C), 128.3 d (3 × C), 128.2 d (3 × C), 128.0 d (2 × C), 127.9 d (3 × C), 127.7 d (2 × C), 127.6 d (3 × C), 127.4 d (2 × C), 127.3 d (3 × C), 127.2 d (2 × C), 123.2 d (arom. C, Bzl, Pht); 135.5 d (C-2″); 116.0 t (C-3″); 97.1 d (C-1′); 96.7 d (C-1); 79.5 d (C-4′); 79.0 d (C-3′); 78.1 d (C-3); 74.8 t (2 × C) (CH$_2$–Ph); 74.9 d (C-5′); 74.7 d (C-4); 73.3 t (CH$_2$–Ph); 72.6 t (C-1″); 72.6 t (CH$_2$–Ph); 70.6 d (C-5); 69.5 t (CH$_2$–Ph); 68.2 t (C-6′); 68.1 t (C-6); 56.6 d (C-2′); 52.1 d (C-2); 23.3 q (NHCO**C**H$_3$). For $C_{60}H_{62}N_2O_{12}$ calculated: relative molecular mass 1 003.2, monoisotopic mass 1 002.4. FAB MS, *m/z*: 1 003.4 $[M + H]^+$. For $C_{60}H_{62}N_2O_{12}$ (1 003.2) calculated: 71.83% C, 6.22% H, 2.79% N; found: 71.63% C, 6.29% H, 2.77% N.

Method B. Compound **5** (18.7 g, 30 mmol) was dried in an apparatus with a septum at 1.32 Pa and room temperature for 8 h. The apparatus was flushed with argone (2 ×) and dry dichloromethane (100 ml) was added through the septum. After dissolution, the mixture was cooled to 0 °C and a solution of 1 M bromine in dichloromethane (30 ml, 30 mmol) was added through the septum. The mixture was stirred at 0° C for 45 min. In the same apparatus, the solvents were evaporated in vacuo (water pump) with exclusion of moisture and the residue was coevaporated with dry toluene (3 × 70 ml) at 133.3 Pa, added through the septum, and dried at 1.32 Pa for 1 h. The solid foam obtained was dissolved by addition of dry dichloromethane (55 ml) through the septum and the resulting solution of glycosylbromide **6** was immediately used for the condensation with **7**.

A mixture of compound **7** (8.83 g, 20 mmol) and silver triflate (7.71 g, 30 mmol) was dried in apparatus equipped with a septum at room temperature and 1.32 Pa for 6 h. The apparatus was flushed with argone (2 ×) and dry dichloromethane (55 ml) was added through the septum. After dissolution, the mixture was cooled to –45 °C and the solution of glycosyl donor **6** (see above) was added through the septum under stirring during 1 h. The mixture was stirred for another 1 h at –45 °C and 30 min at –20 °C. Pyridine (9 ml) was added at –20 °C and after warming to room temperature the mixture was diluted with chloroform (300 ml) and filtered. The filtrate was washed with 0.5 M HCl (3 × 200 ml), saturated solution of $NaHCO_3$ (3 × 200 ml) and water (2 × 200 ml), dried over anhydrous magnesium sulfate and evaporated. The residue was worked up by the same procedure as given in *A* to give 14.5 g (72%) of compound **8**, identical (m.p., $[\alpha]_D$, IR and NMR spectra) with compound prepared by method *A*. For $C_{60}H_{62}N_2O_{12}$ (1 003.2) calculated: 71.83% C, 6.22% H, 2.79% N; found: 71.70% C, 6.32% H, 2.69% N.

Benzyl 2-Acetamido-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-propylamino-β-D-glucopyranosyl)-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**9**)

Method A. Compound **8** (2.0 g, 2 mmol) was refluxed in a mixture of ethanol–hydrazine hydrate (2 : 1, 50 ml) for 5 h. The mixture was concentrated *in vacuo*, coevaporated with toluene (3 × 30 ml) and the residue was dissolved in small amount of methanol. The pH of the solution was adjusted to 5 with formic acid and the solution was poured on a column of Dowex 50 in the pyridinium form (80 ml). The column was washed with methanol (600 ml) and the product was desorbed with 5% solution of triethylamine in methanol (600 ml). The eluate was evaporated to give 1.46 g of solid residue, which was crystallized from toluene–petroleum ether. Yield 1.05 g (60%) of compound **9**; m.p. 145–147 °C, $[\alpha]_D$ +68° (*c* 0.4, chloroform). IR spectrum (tetrachloromethane): 3 452 (N–H, amide); 3 090, 3 067, 3 032, 3 010 (C–H, Bzl); 1 691 (amide I); 1 608, 1 498, 1 454 (arom. ring, Bzl); 1 505 (amide II). ^{1}H NMR spectrum: 7.16–7.36 m, 25 H (arom. H, Bzl); 5.54 d, 1 H, *J* = 9.2 (N**H**COCH$_3$); 4.94 d, 1 H, *J* = 3.8 (H-1); 4.93 d, 1 H, *J* = 11.1 (CH$_2$–Ph); 4.75 d, 1 H, *J* = 10.9 (CH$_2$–Ph); 4.68 d, 1 H, *J* = 10.9 (CH$_2$–Ph); 4.68 d, 1 H, *J* = 11.7 (CH$_2$–Ph); 4.68 d, 1 H, *J* = 11.1

(CH_2–Ph); 4.66 d, 1 H, *J* = 12.1 (CH_2–Ph); 4.60 d, 1 H, *J* = 11.7 (CH_2–Ph); 4.53 d, 1 H, *J* = 12.0 (CH_2–Ph); 4.49 d, 1 H, *J* = 12.1 (CH_2–Ph); 4.45 d, 1 H, *J* = 12.0 (CH_2–Ph); 4.28 d, 1 H, *J* = 8.0 (H-1′); 4.25 ddd, 1 H, *J* = 3.8, 9.2, 10.6 (H-2); 4.05 dd, 1 H, *J* = 8.9, 9.8 (H-4); 3.95 dd, 1 H, *J* = 3.6, 11.0 (H-6b); 3.79 ddd, 1 H, *J* = 1.8, 3.6, 9.8 (H-5); 3.77 dd, 1 H, *J* = 9.1, 9.7 (H-4′); 3.67 dd, 1 H, *J* = 1.8, 11.0 (H-6a); 3.66–3.75 m, 2 H (H-6a′ and H-6b′); 3.53 dd, 1 H, *J* = 8.9, 10.6 (H-3); 3.36 dt, 2 H, *J* = 6.8, 6.8, 8.9 (NHC**H**$_2$$CH_2CH_3$); 3.29 ddd, 1 H, *J* = 2.3, 3.3, 9.7 (H-5′); 3.28 dd, 1 H, *J* = 9.1, 9.8 (H-3′); 2.77 dd, 1 H, *J* = 8.0, 9.8 (H-2′); 1.95 s, 3 H (NHCOC**H**$_3$);1.38–1.52 m, 2 H ($NHCH_2$C**H**$_2CH_3$); 0.78 t, 3 H, *J* = 7.3 ($NHCH_2CH_2$C**H**$_3$). ^{13}C NMR spectrum: 169.6 s (NH**C**OCH_3); 138.5 s, 138.3 s, 138.2 s, 138.1 s, 137.3 s (5 × arom. C, Bzl); 128.5 d (4 × C), 128.4 d (3 × C), 128.3 d (2 × C), 128.0 d (2 × C), 127.8 d (2 × C), 127.7 d (12 × C) (arom. C, Bzl); 102.9 d (C-1′); 97.1 d (C-1); 85.1 d (C-3′); 78.4 d (C-4, C-4′); 75.1 d (C-5′); 74.6 t (CH_2–Ph); 73.4 t (CH_2–Ph); 73.3 t (CH_2–Ph); 73.0 t (CH_2–Ph); 71.2 d (C-3); 70.0 d (C-5); 69.7 t (CH_2–Ph); 68.8 t (C-6′); 68.3 t (C-6); 57.6 d (C-2′); 52.0 d (C-2); 23.4 q (NH**C**OCH_3); 23.3 t (C-2″); 17.7 t (C-1″); 10.5 q (C-3″). For $C_{52}H_{62}N_2O_{10}$ calculated: relative molecular mass 875.1, monoisotopic mass 874.4. FAB MS, *m/z*: 875.3 $[M + H]^+$. For $C_{52}H_{62}N_2O_{10}$ (875.1) calculated: 71.37% C, 7.14% H, 3.20% N; found: 71.28% C, 7.08% H, 3.23% N.

Method B: Compound **8** (1.0 g, 1 mmol) was heated in a solution of hydrazine acetate (1.84 g, 20 mmol) in ethanol (18 ml) at 80 °C for 9 h. The mixture was concentrated *in vacuo*, coevaporated with toluene (3 × 20 ml) and the residue was taken between chloroform (100 ml) and water (20 ml). The organic layer was separated, washed with water (2 × 20 ml), dried over anhydrous magnesium sulfate and evaporated. The residue was worked up, using the same procedure as in method *A*, to give 535 mg (61%) of compound **9**, identical (m.p., $[\alpha]_D$, IR and NMR spectra) with compound prepared by method *A*. For $C_{52}H_{62}N_2O_{10}$ (875.1) calculated: 71.37% C, 7.14% H, 3.20% N; found: 71.21% C, 7.12% H, 3.19% N.

Benzyl 2-Acetamido-4-*O*-(2-amino-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**10**)

A mixture of compound **11** (1.745 g, 2 mmol) and chlorotris(triphenylphosphine)rhodium(I) (200 mg, 0.22 mmol) was refluxed in a mixture of ethanol–toluene–water (7 : 3 : 1, 80 ml) for 10 h under stirring. Formic acid was added (2 ml) and the mixture was refluxed for another 1 h. After cooling, the mixture was filtered and the filtrate was evaporated. The residue was dissolved in a small amount of methanol, pH of the solution was adjusted to 5 with formic acid and the solution was poured on a column of Dowex 50 in the pyridinium form (60 ml). The column was washed with methanol (600 ml) and the product desorbed with 5% triethylamine in methanol (600 ml). The eluate was evaporated and the residue coevaporated with toluene (3 × 20 ml). Crystallization of the residue from a mixture toluene–petroleum ether afforded 1.14 g (68%) of product **10**; m.p. 170–175 °C, $[\alpha]_D$ +60° (*c* 0.4, chloroform). IR spectrum (tetrachloromethane): 3 455 (N–H, amide); 3 090, 3 066, 3 033, 3 008 (C–H, Bzl); 1 688 (amide I); 1 648 (NH_2); 1 608, 1 587, 1 498, 1 454 (arom. ring, Bzl); 1 507 (amide II). ^{1}H NMR spectrum: 7.26–7.35 m, 25 H (H arom., Bzl); 5.60 d, 1 H, *J* = 8.4 (N**H**$COCH_3$); 5.03 d, 1 H, *J* = 3.6 (H-1); 4.93 d, 1 H, *J* = 11.3 (CH_2–Ph); 4.76 d, 1 H, *J* = 10.9 (CH_2–Ph); 4.72 d, 1 H, *J* = 11.8 (CH_2–Ph); 4.68 d, 1 H, *J* = 11.3 (CH_2–Ph); 4.62 d, 1 H, *J* = 12.1 (CH_2–Ph); 4.56 d, 1 H, *J* = 11.9 (CH_2–Ph); 4.56 d, 1 H, *J* = 11.8 (CH_2–Ph); 4.53 d, 1 H, *J* = 10.9 (CH_2–Ph); 4.47 d, 1 H, *J* = 12.1 (CH_2–Ph); 4.47 d, 1 H, *J* = 11.9 (CH_2–Ph); 4.15 d, 1 H, *J* = 8.1 (H-1′); 4.13 ddd, 1 H, *J* = 3.6, 8.4, 10.6 (H-2); 3.86 ddd, 1 H, *J* = 2.0, 4.3, 9.9 (H-5); 3.78 dd, 1 H, *J* = 8.4, 10.6 (H-3); 3.77 dd, 1 H, *J* = 4.3, 10.0 (H-6a); 3.70 dd, 1 H, *J* = 2.0, 10.0 (H-6b); 3.70 dd, 1 H, *J* = 2.3, 10.7 (H-6b′); 3.65 dd, 1 H, *J* = 5.1, 10.7 (H-6a′); 3.64 dd, 1 H, *J* = 8.4, 9.9 (H-4); 3.61 dd, 1 H, *J* = 9.0, 9.9 (H-4′); 3.49 ddd, 1 H, *J* = 2.3, 5.1, 9.9 (H-5′); 3.30 dd, 1 H, *J* = 9.0, 10.0 (H-3′); 2.78 dd, *J* = 8.1, 10.0 (H-2′); 1.97 s,

3 H (NHCOC**H**$_3$). ^{13}C NMR spectrum: 170.1 s (NH**C**OCH$_3$); 138.2 s (2 × C), 137.7 s (2 × C), 137.3 s, 128.6 d (2 × C), 128.5 d (4 × C), 128.4 d (5 × C), 127.9 d (10 × C), 127.8 d (2 × C), 127.7 d (2 × C) (30 × C, arom. C, Bzl); 104.3 d (C-1′); 96.7 d (C-1); 84.7 d (C-3′); 81.3 d (C-4); 78.2 d (C-4′); 75.4 t (CH$_2$–Ph); 74.8 d (C-5′); 74.8 t (CH$_2$–Ph); 73.5 t (CH$_2$–Ph); 73.3 t (CH$_2$–Ph); 70.3 d (C-3); 69.9 d (C-5); 69.8 t (CH$_2$–Ph); 68.7 t (2 × C) (C-6 and C-6′); 57.0 d (C-2′); 53.3 d (C-2); 23.4 q (NHCO**C**H$_3$). For $C_{49}H_{56}N_2O_{10}$ calculated: relative molecular mass 833.0, monoisotopic mass 832.4. FAB MS, *m/z*: 833.5 $[M + H]^+$. For $C_{49}H_{56}N_2O_{10}$ (833.0) calculated: 70.65% C, 6.77% H, 3.36% N; found: 70.47%C, 6.80% H, 3.27% N.

Benzyl 2-Acetamido-3-*O*-allyl-4-*O*-(2-amino-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**11**)

Sodium borohydride (3.03 g, 80 mmol) was gradually added during 2 h to a stired solution of compound **8** (10.03 g, 10 mmol) in a mixture of propan-2-ol–water (6 : 1, 200 ml) at room temperature and the stirring was continued for another 2 h. The solvents were evaporated and the residue was coevaporated with toluene (3 × 60 ml), dissolved in chloroform (350 ml) and extracted with water (3 × 100 ml). The organic phase was dried over anhydrous magnesium sulfate, filtered and evaporated. The solid residue was dissolved in a mixture of toluene–acetic acid (6 : 1, 70 ml) and heated at 85 °C for 5 h. The solvents were evaporated, the residue coevaporated with toluene and dissolved in a small amount of methanol. The pH of the solution was adjusted to 5 with formic acid and the solution was poured on a column of Dowex 50 in the pyridinium form (100 ml). The column was washed with methanol (800 ml) and the product was desorbed with 5% solution of triethylamine in methanol (800 ml). Evaporation of the eluate afforded 6.1 g (70%) of a solid residue which was crystallized from a mixture of toluene and petroleum ether. Yield 4.9 g (56%) of compound **11**; m.p. 139 °C, $[\alpha]_D$ +63° (*c* 0.2, chloroform). IR spectrum (tetrachloromethane): 3 451 (N–H, amide); 3 395, 1 646 (NH$_2$); 3 090, 3 067, 3 032 (C–H, Bzl); 1 691 (amide I); 1 607, 1 498, 1 454 (arom. ring, Bzl); 1 506 (amide II). ^{1}H NMR spectrum: 7.24–7.35 m, 25 H (arom. H, Bzl); 5.80 dddd, 1 H, *J* = 5.1, 6.1, 10.6, 17.0 (H-2″); 5.51 d, 1 H, *J* = 9.3 (N**H**COCH$_3$); 5.15 dq, 1 H, *J* = 3 × 1.7, 17.0 (H-3a″); 5.00 ddt, 1 H, *J* = 1.2, 1.2, 2.0, 10.6 (H-3b″); 4.96 d, 1 H, *J* = 3.7 (H-1); 4.75 d, 1 H, *J* = 11.7 (CH$_2$–Ph); 4.68 d, 1 H, *J* = 12.2 (CH$_2$–Ph); 4.68 d, 1 H, *J* = 12.0 (CH$_2$–Ph); 4.68 d, 1 H, *J* = 11.7 (CH$_2$–Ph); 4.66 d, 1 H, *J* = 12.0 (CH$_2$–Ph); 4.60 d, 1 H, *J* = 11.7 (CH$_2$–Ph); 4.59 d, 1 H, *J* = 12.0 (CH$_2$–Ph); 4.53 d, 1 H, *J* = 12.0 (CH$_2$–Ph); 4.50 d, 1 H, *J* = 12.2 (CH$_2$–Ph); 4.46 d, 1 H, *J* = 11.7 (CH$_2$–Ph); 4.41 ddt, 1 H, *J* = 1.5, 1.5, 5.1, 12.9 (H-1a″); 4.27 d, 1 H, *J* = 7.8 (H-1′); 4.23 ddd, 1 H, *J* = 3.7, 9.0, 10.5 (H-2); 4.05 dd, 1 H, *J* = 8.9, 9.9 (H-4); 4.00 ddt, 1 H, *J* = 1.5, 1.5, 6.1, 12.9 (H-1b″); 3.95 dd, 1 H, *J* = 3.7, 11.0 (H-6a′); 3.78 ddd, 1 H, *J* = 1.7, 3.7, 9.8 (H-5′); 3.71–3.74 m, 2 H (H-6); 3.70 t, 1 H, *J* = 9.5 (H-3′); 3.67 dd, 1 H, *J* = 1.7, 11.0 (H-6b′); 3.56 dd, 1 H, *J* = 8.9, 10.5 (H-3); 3.29 dt, 1 H, *J* = 2.8, 2.8, 9.9 (H-5); 3.28 bt, 1 H, *J* = 9.5 (H-4′); 2.77 dd, 1 H, *J* = 7.8, 10.0 (H-2); 1.94 s, 3 H (NHCOC**H**$_3$). ^{13}C NMR spectrum: 169.7 s (NH**C**OCH$_3$); 138.6 s, 138.4 s, 138.3 s, 138.2 s, 137.3 s (Ph); 135.8 d (C-2″); 128.6 d, 128.5 d (2 × C), 128.4 d (3 × C), 128.3 d (2 × C), 128.1 d (3 × C), 127.8 d (6 × C), 127.7 d (7 × C), 127.5 d (Ph); 115.9 t (C-2″); 103.1 d (C-1′); 97.1 d (C-1); 85.0 d (C-5); 78.4 d (C-3); 78.5 d (C-3′); 75.8 d (C-4); 75.2 t (**C**H$_2$Ph); 75.2 d (C-4′); 74.6 t (**C**H$_2$Ph); 73.5 t (**C**H$_2$Ph); 73.3 t (**C**H$_2$Ph); 72.5 t (C-1″); 71.2 d (C-5′); 69.8 t (**C**H$_2$Ph); 68.8 t (C-6); 68.3 t (C-6′); 57.7 d (C-2′); 52.2 d (C-2); 23.3 q (NHCO**C**H$_3$). For $C_{52}H_{60}N_2O_{10}$ calculated: relative molecular mass 873.1, monoisotopic mass 872.4. FAB MS, *m/z*: 873.4 $[M + H]^+$. For $C_{52}H_{60}N_2O_{10}$ (873.1) calculated: 71.53% C, 6.92% H, 3.20% N; found: 71.48% C, 7.00% H, 3.25% N.

Benzyl 2-Acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-3-*O*-allyl-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**12a**)

To a stirred solution of compound **11** (2.62 g, 3.0 mmol) in a mixture of dichloromethane and pyridine (20 : 1, 42 ml) acetic acid anhydride (0.6 ml, 6.2 mmol) was slowly added and the mixture was stirred for 30 min at 0 °C and another 30 min at room temperature. The solvents were evaporated and the residue was coevaporated with toluene (3 × 15 ml). Column chromatography on silica gel (50 g) in chloroform–ethyl acetate (2 : 1) yielded 2.0 g (73%) of crystalline compound **12a**; m.p. 208–214 °C (dec.), $[\alpha]_D$ +54° (*c* 0.4, chloroform); ref.[1]: m.p. 205–209 °C (dec.), $[\alpha]_D$ +53° (*c* 0.2, chloroform). ^{1}H NMR spectrum: 7.24–7.37 m, 25 H (arom. H, Bzl); 5.77 dddd, 1 H, *J* = 4.8, 6.2, 10.5, 17.1 (H-2″); 5.50 d, 2 H, *J* = 9.2 (2 × N**H**COCH$_3$); 5.14 dq, 1 H, *J* = 3 × 1.8, 17.1 (H-3a″); 4.99 ddt, 1 H, *J* = 1.5, 1.5, 1.9, 10.5 (H-3b″); 4.92 d, 1 H, *J* = 3.9 (H-1); 4.79 d, 1 H, *J* = 11.5 (CH$_2$–Ph); 4.75 d, 1 H, *J* = 11.9 (CH$_2$–Ph); 4.74 d, 1 H, *J* = 12.1 (CH$_2$–Ph); 4.66 d, 1 H, *J* = 11.9 (CH$_2$–Ph); 4.58 d, 1 H, *J* = 11.9 (CH$_2$–Ph); 4.58 d, 1 H, *J* = 12.1 (CH$_2$–Ph); 4.57 d, 1 H, *J* = 11.5 (CH$_2$–Ph); 4.51 d, 1 H, *J* = 11.9 (CH$_2$–Ph); 4.49 d, 1 H, *J* = 8.1 (H-1′); 4.45 d, 1 H, *J* = 12.1 (CH$_2$–Ph); 4.41 d, 1 H, *J* = 11.9 (CH$_2$–Ph); 4.41 ddt, 1 H, *J* = 1.4, 1.4, 4.8, 13.2 (H-1a″); 4.19 ddd, 1 H, *J* = 3.9, 9.2, 10.6 (H-2); 3.96 ddt, 1 H, *J* = 1.4, 1.4, 6.2, 13.2 (H-1b″); 3.89 dd, 1 H, *J* = 8.6, 9.9 (H-4); 3.74 dd, 1 H, *J* = 2.2, 10.8 (H-6a′); 3.70 dd, 1 H, *J* = 3.6, 10.8 (H-6b′); 3.67–3.70 m, 3 H (H-5, H-6a and H-2′); 3.63 dd, 1 H, *J* = 8.8, 9.7 (H-4′); 3.54 dd, 1 H, *J* = 8.8, 9.7 (H-3′); 3.50 dd, 1 H, *J* = 8.6, 10.6 (H-3); 3.49 dd, 1 H, *J* = 2.4, 10.8 (H-6b); 3.35 ddd, 1 H, *J* = 2.2, 3.6, 9.7 (H-5′); 1.93 s, 3 H, 1.69 s, 3 H (2 × NHCOC**H**$_3$). ^{13}C NMR spectrum: 169.9 s (NH**C**OCH$_3$); 169.7 s (NH**C**OCH$_3$); 138.5 s, 138.3 s, 138.2 s (2 × C), 137.3 s (Ph); 135.8 d (C-2″), 128.7 d (2 × C), 128.6 d (2 × C), 128.5 d (2 × C), 128.4 d (4 × C), 128.3 d (2 × C), 128.1 d (2 × C), 128.0 d (3 × C), 127.9 d, 127.8 d (3 × C), 127.7 d, 127.6 d (2 × C), 127.5 (Ph); 115.9 t (C-3″); 100.2 d (C-1′); 97.0 d (C-1); 81.8 d (C-3′); 78.5 d (C-4′); 78.4 d (C-3); 76.7 d (C-4); 74.8 d (C-5); 74.6 t (CH$_2$–Ph); 74.5 t (CH$_2$–Ph); 73.6 t (CH$_2$–Ph); 73.4 t (CH$_2$–Ph); 73.0 t (C-1″); 70.7 d (C-5); 69.8 t (CH$_2$–Ph); 68.8 t (C-6′); 68.1 t (C-6); 56.4 d (C-2′); 52.2 d (C-2); 23.4 q (NHCO**C**H$_3$); 23.3 q (NHCO**C**H$_3$). For $C_{54}H_{62}N_2O_{11}$ calculated: relative molecular mass 915.1, monoisotopic mass 914.4. FAB MS, *m/z*: 915.5 $[M + H]^+$. For $C_{54}H_{62}N_2O_{11}$ (915.1) calculated: 70.89% C, 6.82% H, 3.06% N; found: 70.67% C, 6.86% H, 2.96% N.

Benzyl 2-Acetamido-3-*O*-allyl-6-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-stearamido-β-D-glucopyranosyl)-2-deoxy-α-D-glucopyranoside (**12b**)

To a stirred solution of compound **11** (5.24 g, 6.0 mmol) in a mixture of dichloromethane and pyridine (4 : 1, 70 ml), stearoyl chloride (3.03 g, 10 mmol) was added. After 5 h stirring at room temperature, methanol (3 ml) was added and the mixture was stirred for 1 h. The solvents were evaporated and the solid residue was extracted with petroleum ether (3 × 70 ml) to remove methyl stearate. Chromatography of the residue on a silica gel column (120 g) in chloroform–ethyl acetate (3 : 1) and concentration *in vacuo* yielded 5.8 g (85%) of solid product **12b**, $[\alpha]_D$ +43° (*c* 0.5, chloroform); ref.[3]: $[\alpha]_D$ +43° (*c* 0.4, chloroform). For $C_{70}H_{94}N_2O_{11}$ calculated: relative molecular mass 1 139.5, monoisotopic mass 1 138.7. FAB MS, *m/z*: 1 139.8 $[M + H]^+$. For $C_{70}H_{94}N_2O_{11}$ (1 139.5) calculated: 73.78% C, 8.31% H, 2.45% N; found: 72.52% C, 8.37% H, 2.39% N.

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REFERENCES

1. Farkas J., Ledvina M., Brokes J., Jezek J., Zajicek J., Zaoral M.: *Carbohydr. Res.* **1987**, *163*, 63.
2. Ledvina M., Farkas J., Zajicek J., Jezek J., Zaoral M.: *Collect. Czech. Chem. Commun.* **1989**, *54*, 2784.
3. Ledvina M., Saman D., Jezek J.: *Collect. Czech. Chem. Commun.* **1992**, *57*, 579.
4. Hribalova V., Vacek A., Toman M., Horavova P., Ledvina M., Jezek J. in: *Peptides 1994, Proc. 23rd Eur. Pept. Symp., September 4–10, 1994, Braga, Portugal* (H. L. S. Maia, Ed.), p. 847. ESCOM, Leiden 1995.
5. Turanek J., Zaluska D., Vacek A., Hofer M., Ledvina M., Jezek J. in: *Peptides 1996, Proc. 24th Eur. Pept. Symp., September 4–10, 1996, Edinburgh, U.K.* (R. Ramage and R. Epton, Eds). ESCOM, Leiden 1998.
6. Durette P. L., Meitzner E. P., Shen T. Y.: *Carbohydr. Res.* **1979**, *77*, C1.
7. Lonn H.: *Carbohydr. Res.* **1985**, *139*, 105.
8. Barker B. R., Joseph J. P., Schaub R. E., Williams J. H.: *J. Org. Chem.* **1954**, *19*, 1786.
9. Best W. M., Dunlop R. W., Stick R. V., White S. T.: *Aust. J. Chem.* **1994**, *47*, 433.
10. Kukolja S., Lammert R. S.: *J. Am. Chem. Soc.* **1975**, *97*, 5582.
11. Dasgupta F., Garegg P. J.: *J. Carbohydr. Chem.* **1988**, *7*, 701.
12. Lemieux R. U., Tkeda Y., Chung B. Y.: *ACS Symp. Ser.* **1976**, *39*, 90.
13. Lonn H.: *J. Carbohydr. Chem.* **1987**, *6*, 301.
14. Hashimoto H., Abe Y., Horito S., Yoshimura J.: *J. Carbohydr. Chem.* **1989**, *8*, 307.
15. Bergmann M., Zervas L.: *Chem. Ber.* **1931**, *64*, 975.
16. Garrohelion F., Merzouk A., Guibe F.: *J. Org. Chem.* **1993**, *58*, 6109.
17. Lemaire-Audoire S., Savignac M., Genet J. P., Bernard J. M.: *Tetrahedron Lett.* **1995**, *36*, 1267.
18. Osby J. O., Martin M. G., Ganem B.: *Tetrahedron Lett.* **1984**, *25*, 2093.