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Carbohydrates as Nucleophiles in Conjugate Addition for Preparation of Muramic Acid Analogues

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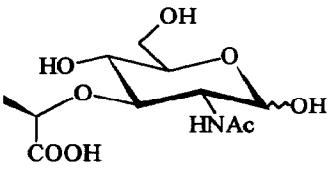
Abstract: Benzyl 2-acetamido-6-O-benzyl-3-O-[(S)-1-carboxy-isopropyl]-α-D-glucopyranoside (**15**) was synthesized stereoselectively by conjugate addition reaction, starting from benzyl 2-acetamido-6-O-benzyl-2-deoxy-α-D-glucopyranoside (**9**) and crotonic acid ethyl ester under phase transfer conditions. The dipeptide L-Ala-D-Glu(OMe)OMe was coupled to **15** to give compuond **25** an analogue of the adjuvant active muramyl dipeptide (MDP)

INTRODUCTION

N-Acetylmuramic acid (**1**) is a constituent of the polymer peptidoglycan which builds up the bacterial cell wall[1]. The structure of peptidoglycan consists of polysaccharide chains with alternating β(1→4) glycosidically linked units of N-acetylglucosamine and N-acetylmuramic acid which are crosslinked via peptide chains. Fractions of bacterial cell walls show marked adjuvant activity, i.e. by coadministration with an antigen a much higher titer of antibodies is observered than with just the antigen alone. The minimal adjuvant-active structure capable of replacing whole bacterial cells in this so called "Freund's complete adjuvant" for increasing production of antibodies is N-acetylmuramyl-L-alanyl-D-isoglutamine (**2**; muramyldipeptide, MDP)[2].

The possibility to obtain molecules for modulation of the immunosystem made MDP and its derivatives a target for many synthetic and biological studies[3]. Several hundred derivatives have been synthesized and tested to date. The main biological activities of MDP and its derivatives are:

- adjuvant activity
- induction of delayed hypersensitivity against an antigen
- stimulation of non-specific resistance against bacterial, viral and parasite infections
- anti-tumor activity
- somnogenic activity.



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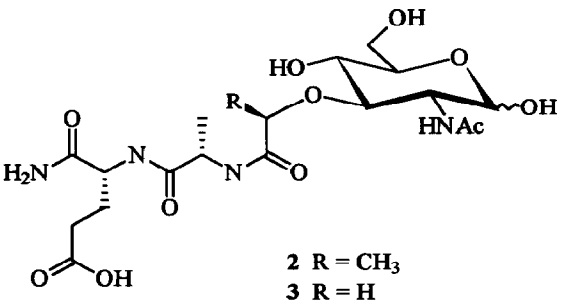
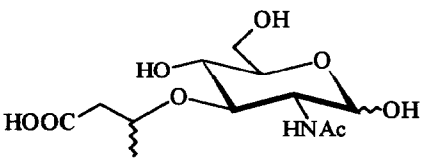


2 R = CH_3
3 R = H
4 R = $CH_2CH_2CH_3$

Unfortunately most of the derivatives induce several undesired side-effects such as pyrogenicity, transient leukopenia, sensitization to endotoxines, and induction of arthritis or granulomas. Thus, new derivatives with hopefully less side-effects remain to be of major interest. Variations have mainly been pursued in the peptide chain and by alternating the lipophilicity either via 6-O-acyl derivatives or by adding a lipophilic moity to the peptide residue[3]. Two interesting compounds are Nor-MDP (**3**) and 3′-n-propyl-MDP (**4**) which are modified in the lactyl residue[4]. They still show biological activity but are less toxic than most other derivatives. This promted us to attempt syntheses of N-acetylhomomuramic acids, analogues of muramic acid with a novel modification in the former lactyl residue such as (**5**).



5

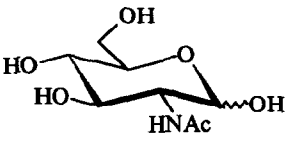
Compounds of this type should be readily accessible via conjugate addition reaction with a crotonic acid derivative as acceptor molecule and a properly protected glucosamine as the nucleophile. There are only a few examples of carbohydrates serving as nucleophiles in conjugate additions leading to the formation of ether bonds. In fact, merely intramolecular reactions of this type have been described to date, forming five and six membered rings[5,6].

Therefore two main stategies appeared to be promising either employing an intramolecular reaction with **12** serving as decisive precursor molecule or an intermolecular approach with for example compounds **7** or **9** as nucleophiles.

RESULTS AND DISCUSSION

Intramolecular strategy: Starting from N-acetylglucosamine (**6**), the benzylidenated glycoside **7** was prepared according to literature[7]. Etherification of **7** with allylbromide and sodium hydride in dioxane gave **8**[8] in quantitative yield. Reductive opening of the benzylidene ring with sodium cyanoborohydride in THF yielded

60% of **10**[9], from which the ester **11** was prepared with crotyl chloride and pyridine in 55% yield. Selective deallylation was achieved by using palladium on charcoal (10%), p-toluene sulfonic acid as acidic catalyst, and methanol as the solvent[10] and led to **12** in 85% yield.



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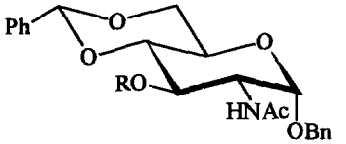
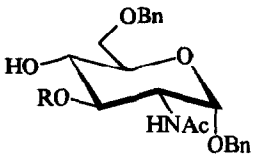
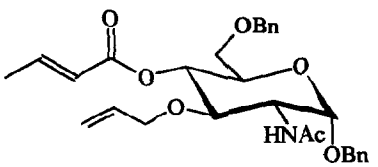


7 R = H
8 R = All

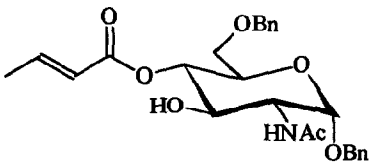


9 R = H
10 R = All

10



11



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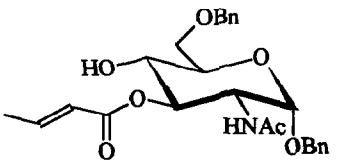

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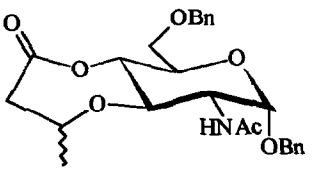
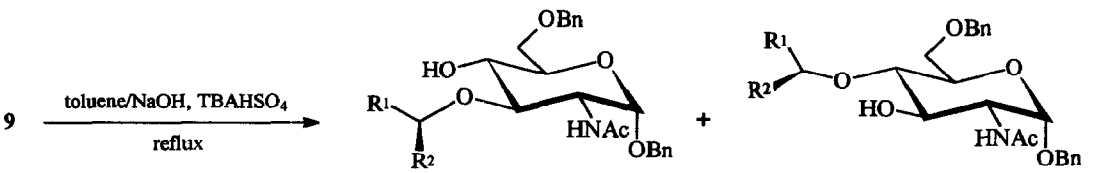


13

Although the formation of seven membered cyclic ethers via intramolecular conjugate addition of unsaturated esters was reported[11], it was not possible to synthesize, under various conditions, the bicyclic derivative **13**. The only compounds obtained were the ester migration product **14** and unreacted **12** in addition to hydrolysis or decomposition products. The structure of **14** was proved by ^{1}H-NMR due to the upfield shift for H-4 (approximately 1.15 ppm) and the downfield shift for H-3 (1.34 ppm). The formation of a five membered transition state, leading to **14**, seems to be highly favoured, since not even traces of **13** could be detected.

Intermolecular strategy: Starting from **7**, the diol component **9**[12] was obtained by reductive opening of the benzylidene ring with sodium cyanoborohydride[13] in 62% yield. The reaction of **9** with a considerable excess of crotonic acid ethylester under phase transfer conditions (toluene/20% NaOH, reflux, tetrabutylammonium hydrogensulfate) gave the two possible regioisomers as a mixture of diastereomers (**15,16** and **17,18**) in 63% and 20% yield, respectively. **17** and **18** were identified as their methyl esters **20** and **21**; the ratio of diastereomers was 1:1, determined by ^{1}H-NMR, for both the 3- and the 4-regioisomers.

Under milder conditions (CH_2Cl_2/20% NaOH, r.t., tetrabutylammonium hydrogensulfate) **15** was obtained as single diastereomer in 76% yield together with a diastereomeric mixture of the regioisomers **17** and **18** as side products (ratio 1 1, 15% yield, identified as methyl esters).



63%

15 $R^1 = CH_2COOH$, $R^2 = CH_3$

16 $R^1 = CH_3$, $R^2 = CH_2COOH$

20%

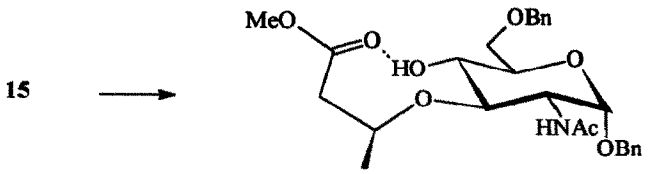
17 $R^1 = CH_2COOH$, $R^2 = CH_3$

18 $R^1 = CH_3$, $R^2 = CH_2COOH$

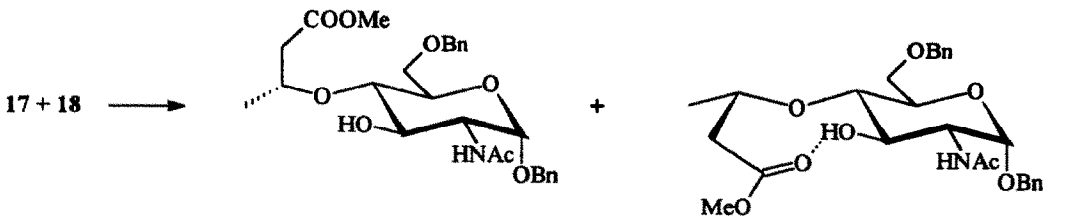
9 $\xrightarrow[\text{r.t.}]{CH_2Cl_2/NaOH,\ TBAHSO_4}$ **15** (76%) and **17** + **18** (15%)

The stereochemistry in the ether side chain of **15** could be determined by transforming **15** into its methylester **19** and ^{1}H-NMR spectroscopy. The formation of the ether linkage at C-3 in the sugar is proven by an upfield shift for H-3 by 0.26 ppm. A dublett at rather low field (5.20 ppm), which disappears when D_2O or

MeOD are added, is assessed to 4-OH. The chemical shift indicates an intramolecular hydrogen bridge between the carbonyl group of the ester and the the 4-OH proton of the sugar.

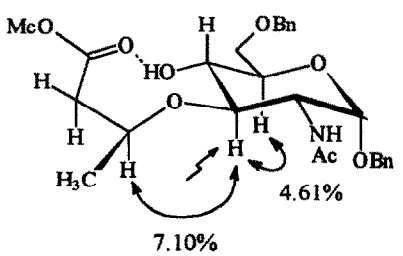


19



20 21

Since only one conformer could be detected in $CDCl_3$ solution, it was possible to perform NOE experiments with **19** to obtain information about the absolute configuration of the newly formed stereocenter in the side chain. Irradiation of H-3 gave a strong effect on the proton of the CH-group (7.10%) and clear effects on H-5 (4.61%), the protons of CH_3 (2.55%) and the amide proton of the acetamido group (2.90%), indicating that the latter is located under the plane of the sugar ring. The crossexperiment with irradiation of the CH-group gave an even stronger effect with H-3 (7.68%), and clear effects with the protons of CH_3 (4.93%) and the axial proton of the CH_2-group (3.46%). These results clearly prove the (S)-configuration at C-3 of the side chain.



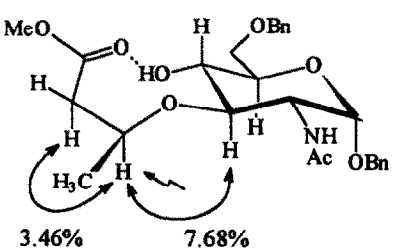
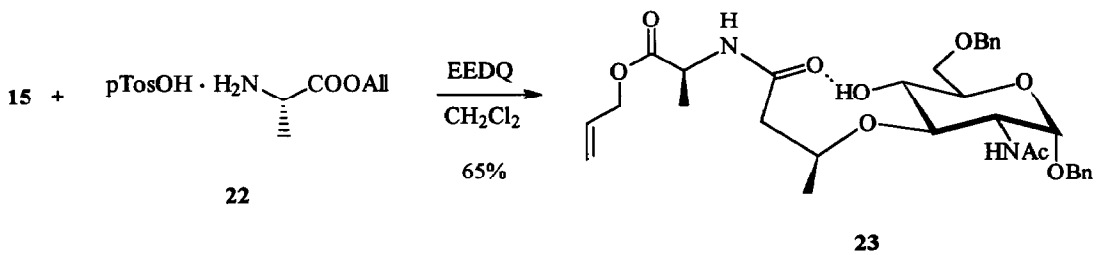


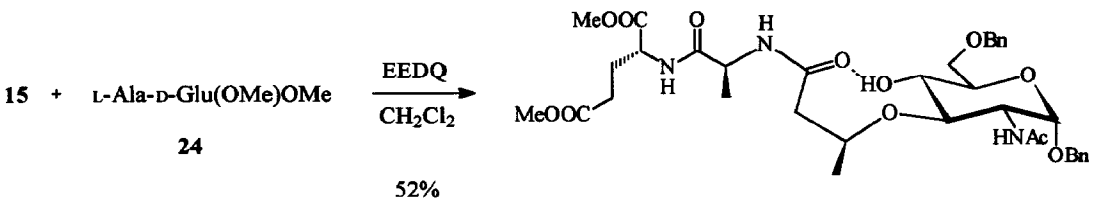
Figure 1: NOE-effects in **19**

The same effect of an intramolecular hydrogen bridge forcing the conformation towards a nine membered ring can be observed in **21**. In contrast, in **20** a possible hydrogen bridge seems to be too weak to force the methyl group into the unfavoured axial position of the ring. This is supported by the NMR spectrum of the diastereomeric mixture of **20** and **21**, in which two different dubletts, each with an integral of approximatly 0.5 protons, can be observed. One at 5.06 ppm belongs to the hydrogen-bridged OH-4 in **21**, the other at 3.02 ppm being the free hydroxy group in **20**.

As an explanation for the experimental results the higher nucleophilicity of the 3-OH group of the N-acetylglucosamine residue[14], could account for the regioselectivity. The stereoselectivity in the formation of the novel chiral center may be explained by an interaction of the amide proton with the carbonyl group in the crotonic acid ethylester. This would induce a position of the ester above the ring of the sugar, thus allowing an attack of the O-3 from below, which in turn would lead to the observed (S)-configuration in **15**. Apparently, this interaction is disturbed at higher temperature and no stereoselectivity is found under reflux in toluene with NaOH In case of the regioisomers **17** and **18** no such interaction is possible and no stereoselectivity in the addition could be observed. Thus, under both reaction conditions hydrolysis of the intermediate ethyl esters takes place.



22 **23**



24 52% **25**

Starting from **15** it was possible to achieve coupling with the toluenesulfonic acid salt of L-alanyl allylester (**22**)[15] with ethyl-2-ethoxy-1,2-dihydroquinoline-1-carboxylate (EEDQ) as coupling reagent[16] to form the N-acetyl-(S)-homomuramyl-L-alanyl allylester derivative **23** in 65% yield. Similar to **19** the ^{1}H-NMR spectrum shows a dublett at high field (5.43 ppm, J = 3.6 Hz), which disappears by addition of D_2O or MeOD, indicating an intermolecular hydrogen bridge.

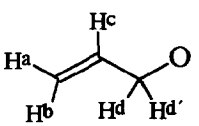
In case of N-acetyl muramic acid coupling with the dipeptide L-Ala-D-Glu(OMe)OMe (**24**) gave a fully active and almost apyrogenic MDP derivative[17]. Here the precoursor **15** was condensed with the same dipeptide **24** to give the analogue **25** in 52% yield. Like **19** it shows the typical dublett of a hydrogen bridged proton (5.42 ppm, J = 2.5 Hz).

Syntheses of other derivatives of N-acetyl homomuramic acid with different peptide moieties and potentialy enhanced biological activity as well as investigations on the different coupling methods are under way.

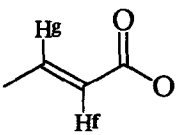
EXPERIMENTAL

General methods - NMR spectra were recorded on Brucker AMX 400 at 400 MHz with $SiMe_4$ as internal standard. Chemical shifts are given in ppm down field from $SiMe_4$ and J values in Hz. NMR assignments were made using standard $^1H,^1H$-COSY experiments. ^1H NMR chemical shifts of overlapping signals were obtained from the center of the cross-peaks in the $^1H,^1H$ COSY spectra. Melting points were taken using a Olympus polarising microscope and are uncorrected. T.l.c. was carried out on silica gel (60 F_{254} Merck) on aluminium foil. Preparative column chromatography was performed on silica gel (60, 230-400 mesh, 0.040-0.063 mm, Merck) using the flash technique. Optical rotations were measured at ~ 20 °C using a Perkin-Elmer Model 241 polarimeter and a 1dm cuvette. Evaporations were carried out at <45 °C under diminished pressure.

Allyl group:



Crotyl group:



Benzyl 2-acetamido-3-O-allyl-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (**8**): Sodium hydride (450 mg, 80% in paraffine) was added to a solution of **7** (3.10 g, 7.7 mmol) in dry dioxane (150 mL). The mixture was refluxed for 30 min, allylbromide was added (0.75 mL, 8.8 mmol) and again heated under reflux for 45 min. The mixture was cooled and water (1mL) was added to destroy excess sodium hydride. The solvent was evaporated chloroform (400 mL) was added and the organic layer extracted with water (3x 100 mL), dried ($MgSO_4$), filtered and concentrated to give crystalline **8** (3.35 g, 99%), pure by t.l.c. and NMR; m.p. 254-256°; $[\alpha]_D^{20}$ +120 (*c* 1, pyridine) – ^1H-NMR ($CDCl_3$): δ = 7.50 - 7.20 (m, 10 H, Ph), 5.84

(ddd, 1 H, H-c, $J_{a,c}$ = 10.2, $J_{b,c}$ = 17.3), 5.58 (d, 0.9 H, NH, $J_{NH,2}$ = 8.7), 5.57 (s, 1 H, PhCH), 5.24 (dd, 1 H, H-b, Jb,a = 1.5), 5,12 (dd, 1 H, H-a), 4.94 (d, 1 H, H-1α, $J_{1,2}$ = 3.6), 4.72 (d, 1 H, Bn, J_{gem} = 11.7), 4.48 (d, 1 H, Bn'), 4.39 (dd, 1 H, H-d, $J_{c,d}$ = 5.6, $J_{d,d'}$ = 13.2), 4.29 (ddd, 1 H, H-2, $J_{2,3}$ = 9.7), 4.24 (dd, 1 H, H-6eq, J_{gem} = 10.2), 4.08 (dd, 1 H, H-d'), 3.89 (ddd, 1 H, H-5, $J_{5,6eq}$ = 4.6, $J_{5,6ax}$ = 9.7, $J_{4,5}$ = 9.4), 3.88 (dd, 1 H, H-3, $J_{3,4}$ = , $J_{2,3}$ = 9.7), 3.80 - 3.63 (m, 2 H, H-4 and H-6ax), 1.92 (s, 3 H, Ac).

Anal. Calcd for $C_{25}H_{29}NO_6$: C 68.32 H 6.65 N 3.19 Found: C 68.19 H 6.68 N 3.19

Benzyl 2-acetamido-6-O-benzyl-2-deoxy-α-D-glucopyranoside (**9**): To a stirred solution of **7** (2.0 g, 5 mmol) and sodium cyanoborohydride (3.0 g) in dry THF (100 mL) a saturated solution of hydrogen chloride in dry diethylether was added at 0°C until gas evaluation ceased and the pH maintained at 1. Stirring was continued for another 30 min. Then the solution was poured onto ice-water (400 mL) and extracted with chloroform (3x 150 mL). The combined organic layers were washed with saturated sodium hydrogen carbonate solution (100 mL), dried ($MgSO_4$), filtered and evaporated to dryness. The residue was subjected to flash chromatography (dichloromethane/methanol 10:1) to give of **9** (1.24 g, 62%); m.p. 183°, $[\alpha]_D^{20}$ +45 (*c* 1, methanol) – ^{1}H-NMR (MeOD): 7.30 - 7.11 (m, 10 H, Ph), 4.76 (d, 1 H, H-1α, $J_{1,2}$ = 3.6), 4.62 (d, 1 H, 1-Bn, J_{gem} = 11.7), 4.50 (bs, 2 H, 6-Bn and 6-Bn'), 4.39 (d, 1 H, 1- Bn'), 3.81 (dd, 1 H, H-2, $J_{2,3}$ = 11.2), 3.74 - 3.55 (m, 4 H, H-3, H-5, H-6, H-6'), 3.31 (dd = t, 1 H, H-4, $J_{3,4}$ = 9.4, $J_{4,5}$ = 9.4), 1.87 (s, 3 H, Ac).

Anal. Calcd for $C_{22}H_{27}NO_6$: C 65.82 H 6.78 N 3.49 Found: C 65.65 H 6.80 N 3.50

Benzyl 2-acetamido-3-O-allyl-6-O-benzyl-2-deoxy-a-D-glucopyranoside (**10**): A stirred solution of **8** (0.88 g, 2.0 mmol) and sodium cyanoborohydride (1.13 g) in dry THF (70 mL) was cooled to 0 °C. A saturated solution of hydrogen chloride in diethylether was added until gas evaluation ceased and the pH maintained at 1. The mixture was stirred for another 30 min, poured on ice-water (200 mL) and extracted with chloroform (3x 100 mL) The organic layer was washed with saturated sodium hydrogencarbonate, dried ($MgSO_4$) and evaporated to dryness. Flash chromatotography of the residue (dichlormethane/acetone 5:1) gave **10** (530 mg, 60%); m.p. 149-150°; $[\alpha]_D^{20}$ +100 (*c* 1, chloroform) – ^{1}H-NMR ($CDCl_3$): δ = 7.40 - 7.25 (m, 10 H, Ph), 5.88 (ddd, 1 H, H-c, $J_{a,c}$ = 10.5, $J_{b,c}$ = 17.0), 5.56 (d, 0.9 H, NH, $J_{NH,2}$ = 9.5), 5.25 (dd, 1 H, H-b, $J_{a,b}$ = 1.5), 5.15 (dd, 1 H, H-a), 4.89 (d, 1 H, H-1, $J_{1,2}$ = 3.5), 4.72 (d, 1 H, 1-Bn, J_{gem} = 11.7), 4.65 (d, 1 H, 6-Bn, J_{gem} = 12.0), 4.56 (d, 1 H, 6-Bn'), 4.46 (d, 1 H, 1-Bn'), 4.25 (dd, 1 H, H-d, J_{gem} = 10.0), 4.21 (m, 1 H, H-2), 4.15 (dd, 1 H, H-d'), 3.81 (m, 1 H, H-5, $J_{4,5}$ = 9.5), 3.77 - 3.66 (m, 3 H, H-4, H-6, H-6'), 3.51 (dd, 1 H, H-3, $J_{2,3}$ = 10.0, $J_{3,4}$ = 8.5), 2.71 (d, 0.8 H, 4-OH, $J_{OH,H-4}$ = 1.5), 1.96 (s, 3 H, Ac).

Anal. Calcd for $C_{25}H_{31}NO_6$: C 68.01 H 7.08 N 3.17 Found: C 67.94 H 7.10 N 3.16

Benzyl 2-acetamido-3-O-allyl-6-O-benzyl-4-O-crotyl-2-deoxy-α-D-glucopyranoside (**11**): **10** (200 mg, 0.45 mmol) was dissolved in dry dichloromethane (10 mL) and cooled to 0 °C. Pyridine (50 µL, 0.6 mmol) and crotylchloride (50 µL, 0.5 mmol) were added. The solution was allowed to warm to room temperature and was stirred for 12 h. It was diluted with dichloromethane (10 mL) and washed with saturated sodium hydrogencarbonate (2x 10 mL) and water (2x 10mL). The aqueous layers were extracted with dichloromethane (15 mL) and the combined organic layers washed with brine (10 mL), dried ($MgSO_4$) and evaporated to dryness. Flash chromatography of the residue (dichloromethane/acetone 10:1, containing 1% triethylamine) yielded **11** (126 mg, 55%); m.p. 112°; $[\alpha]_D^{20}$ +125° (*c* 1, chloroform) – ^{1}H-NMR ($CDCl_3$): δ =

7.40 - 7.22 (m, 10 H, Ph), 7.05-6.93 (m, 1 H, H-g), 5.80 (dd, 1 H, H-f, $J_{f,g}$ = 15.3, $^4J_{f,Me}$ = 1.5), 5.76 (ddd, 1 H, H-c, $J_{a,c}$ =10.7, $J_{b,c}$ = 17.3), 5.54 (d, 0.9 H, NH, $J_{NH,2}$ = 9.2), 5.21-5.05 (m, 3 H, H-a, H-b, H-4), 4.96 (d, 1 H, H-1α, $J_{1,2}$ = 3.6), 4.75 (d, 1 H, 1-Bn, J_{gem} = 11.7), 4.53 (m, 2 H, 6-Bn and 6-Bn'), 4.48 (d, 1 H, 1-Bn'), 4.33 (ddd, 1 H, H-2, $J_{2,3}$ = 9.7), 4.09-3.93 (m, 3 H, H-d, H-d', H-5), 3.70 (dd, 1 H, H-3, $J_{3,4}$ = 10.2), 3.56-3.51 (m, 2 H, H-6 and H-6'), 1.96 (s, 3 H, Ac), 1.88 (d, 3 H, CH_3).

Anal. Calcd for $C_{29}H_{34}NO_7$: C 68.49 H 6.74 N 2.75 Found: C 68.31 H 6.77 N2.75

Benzyl 2-acetamido-6-O-benzyl-4-O-crotyl-2-deoxy-α-D-glucopyranoside (**12**): A solution of **11** (100 mg, 0.2 mmol) in methanol (12 mL) containing 10% palladium on charcoal (80 mg) and toluenesulfonic acid monohydrate (30 mg) was heated under reflux for 2 h. After cooling the mixture was filtrated and evaporated to dryness. Flash chromatography (dichlorometane/acetone 5:1, 1% triethylamine) yielded **12** (foam, 80 mg, 85%); $[\alpha]_D^{20}$ +93.7 (*c* 1, chloroform) – ^{1}H-NMR ($CDCl_3$): δ = 7.40-7.20 (m, 10 H, Ph), 6.97 (m, 1 H, H-g, $J_{f,g}$ =14.3, $J_{g,Me}$ = 7.1), 5,80 (dd, 1 H, H-f, $^4J_{f,Me}$ = 1.5), 5.78 (d, 0.9 H, NH, $J_{NH,2}$ = 8.7), 5.02 (dd, 1 H, H-4, $J_{3,4}$ = 10.2, $J_{4,5}$ = 9.7), 4.96 (d, 1 H, H-1α, $J_{1,2}$ = 3.6), 4.77 (d, 1 H, 1-Bn, J_{gem} = 11.7), 4.57 (d, 1 H, 6-Bn, J_{gem} = 12.2), 4.52 (d, 1 H, 6-Bn'), 4.50 (d, 1 H, 1-Bn'), 4.20 (ddd, 1 H, H-2, $J_{2,3}$ = 10.4), 3.97 (ddd, 1 H, H-5, $J_{5,6ax}$ = 8.1, $J_{5,6eq}$ = 4.1), 3.84 (ddd, 1 H, H-3, $J_{3,4}$ = 10.2, $J_{3,OH}$ = 6.1), 3.55 (m = d, 2 H, H-6ax and H-6eq), 3.14 (d, 0.8 H, 3-OH), 1.96 (s, 3 H, Ac), 1.72 (dd, 3 H, CH_3).

Anal. Calcd for $C_{26}H_{30}NO_7$: C 66.65 H 6.45 N 2.99 Found: C 66.68 H6.47 N 3.01

Benzyl 2-acetamido-6-O-benzyl-3-O-crotyl-2-deoxy-α-D-glucopyranoside (**14**): **12** (20 mg, 0.04 mmol) in dry chloroform (5 mL) containing molecular sieves, were stirred with DBU (20 µL) for 24 h. The reaction mixture was diluted with chloroform (5 mL), filtered and evaporated to dryness. Flash chromatograpy (dichloromethane/aceton 5:1, 1% triethylamine) yielded **14** (foam, 19 mg, 95%); $[\alpha]_D^{20}$+149 (*c* 1, chloroform) – ^{1}H-NMR ($CDCl_3$): δ = 7.40-7.20 (m, 10 H, Ph), 7.02 (m, 1 H, H-g, $J_{f,g}$ = 13.7, $J_{g,Me}$ = 7.0), 5.85 (dd, 1 H, H-f, $^4J_{f,Me}$ = 1.5), 5.76 (d, 0.9 H, NH, $J_{NH,2}$ =9.7), 5.16 (dd, 1 H, H-3, $J_{2,3}$ =10.7, $J_{3,4}$ = 8.7), 4.92 (d, 1 H, H-1 α, $J_{1,2}$ = 3.6), 4.73 (d, 1 H, 1-Bn, J_{gem} = 12.2), 4.62 (d, 1 H, 6-Bn, J_{gem} = 12.2), 4.56 (d, 1 H, 6-Bn'), 4.49 (d, 1 H, 1-Bn'), 4.30 (ddd, 1 H, H-2), 3.90-3.68 (m, 4 H, H-4, H-5, H-6, H-6'), 2.90 (bs, 0.8 H, 4-OH), 1.87 (dd, 3 H, CH_3), 1.85 (s, 3 H, Ac).

Anal. Calcd for $C_{26}H_{30}NO_7$: C 66.65 H 6.45 N 2.99 Found: C 66.47 H 6.46 N 2.99

Benzyl 2-acetamido-6-O-benzyl-3-O-[2(S)-1-carboxy-isopropyl]-α-D-glucopyranoside (**15**): A solution of **9** (100 mg, 0.25 mmol) and tetrabutylammonium hydrogensulfate (100 mg, 0.25 mmol) in dichloromethane (8 mL) was vigorously stirred with 20% sodium hydroxide solution (5 mL). Crotonic acid ethylester (2 mL) was added and stirring continued for 2 days at room temperature. The mixture was diluted with dichloromethane (10 mL) and the layers separated. The aqueous layer was acidified with concentrated hydrochloric acid and extracted with dichloromethane (3x 15 mL). The combined organic layers are washed with water (10 mL), dried ($MgSO_4$) and evaporated to dryness.Flash chromatography (toluene/ethanol/acetic acid 5:1:0.01) yielded of **15** (93 mg, 76%) and a mixture of **17** and **18** (18 mg, 15%). **15**: m.p. 163-164°; $[\alpha]_D^{20}$ +60 (*c* 1, methanol) – ^{1}H-NMR ($CDCl_3$): δ = 7.42-7.20 (m, 10 H, Ph), 5.78 (bd, 0.8 H, NH, $J_{NH,2}$ = 9.7), 4.85 (d, 1 H, H-1α, $J_{1,2}$ = 3.6), 4.78 (d, 1 H, 1-Bn, J_{gem} = 11.7), 4.61 (d, 1 H, 6-Bn, J_{gem} = 12.2), 4.56 (d, 1 H, 6-Bn'), 4.46 (d , 1 H, 1-Bn'), 4.21 (m, 2 H, CH, H-2), 3.97-3.87 (m, 2 H, H-5, H-6), 3.72 (dd, 1 H, H-

6', $J_{5,6'}$ = 7.1, J_{gem} = 10.7), 3.58 (dd, 1 H, H-4, $J_{3,4}$ = 8.7, $J_{4,5}$ = 9.7), 3.43 (dd, 1 H, H-3, $J_{2,3}$ = 10.2), 2.55 (dd, 1 H, CH_2ax, $J_{ax,CH}$ = 10.2, J_{gem} = 17.2), 2.41 (dd = bd, 1 H, CH_2eq), 1.95 (s 3 H, Ac), 1.04 (d, 3 H, CH_3, $J_{CH,Me}$ = 6.1).

Anal. Calcd for $C_{26}H_{33}NO_8$: C 64.05 H 6.82 N 2.87 Found: C 63.97 H 6.84 N 2.87

Benzyl 2-acetamido-6-O-benzyl-3-O-[2(S)-1-carboxy-isopropyl]-a-D-glucopyranoside (**15**) and *Benzyl 2-acetamido-6-O-benzyl-3-O-[2(R)-1-carboxy-isopropyl]-a-D-glucopyranoside* (**16**): A mixture of a solution of **9** (50 mg, 0.13 mmol) and tetrabutylammonium hydrogensulfate (50 mg, 0.13 mmol) in toluene (5 mL), 20% sodium hydroxide solution (2.5 mL) and crotonic acid ethylester (1.5 mL) were heated under reflux for 6 hours. After cooling dichloromethane (15 mL) was added and the organic layer separated. The aqueous layer was acidified with concentrated hydrochloric acid and extracted with dichloromethane. The combined organic layers were washed with water (10 mL), dried ($MgSO_4$) and evaporated to dryness. Flash chromatographie (toluene/ethanol/acetic acid 5:1:0.01) yielded the diastereomeric mixture of **15** and **16** (40 mg, 63%) and the mixture of **17** and **18** (13 mg). Mixture of **15** and **16**, ^{1}H-NMR ($CDCl_3$), characteristic signals: 7.40-7.22 (m, 10 H, Ph), 4.94 (bd, 1 H, H-1α, $J_{1,2}$ = 3.6), 4.75 and 4.48 (d, 1 H, 1-Bn, J_{gem} = 11.7), 4.63 (m, 2 H, 6-Bn), 2 62-2.27 (m, 2 H, CH_2), 1.97 (s, 3 H, Ac), 1.27 and 1.12 (d, 1.5 H, CH_3, $J_{CH,Me}$ = 6.1).

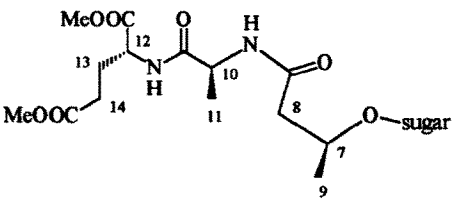
Benzyl 2-acetamido-6-O-benzyl-4-O-[2(R)-1-carboxy-isopropyl]-a-D-glucopyranoside (**17**) and *Benzyl 2-acetamido-6-O-benzyl-4-O-[2(S)-1-carboxy-isopropyl]-a-D-glucopyranoside* (**18**): Reaction conditions see above. Mixture of **17** and **18**, ^{1}H-NMR ($CDCl_3$), characteristic signals: 7.40-7.20 (m, 10 H, Ph), 4.98 (bd, 1 H, H-1α, $J_{1,2}$ =3.6), 4.76-4.45 (m, 4 H, Bn), 2.55-2.30 (m, 2 H, CH2), 1.96 (s, 3 H, Ac), 1.25 and 1 10 (d, 1.5 H, CH3, $J_{CH,Me}$ =6.1)

Benzyl 2-acetamido-6-O-benzyl-3-O-[2(S)-1-carboxymethyl-isopropyl]-a-D-glucopyranoside (**19**): **15** (90 mg, 0.18 mmol) was dissolved in dry methanol (50 mL). A saturated solution of hydrochloric acid in dry ethylether (5 mL) was added and the solution stirred over night. The solvent was evaporated, the residue dissolved in dichloromethane (20 mL), washed with saturated sodium hydrogencarbonate solution, dried ($MgSO_4$) and filtered. Evaporation of the solvent yielded **19** (90 mg, 98%); m.p. 137°, $[\alpha]_D^{20}$ +44 (*c* 1.5, chloroform) – ^{1}H-NMR ($CDCl_3$): δ = 7.40-7.20 (m, 10 H, Ph), 5.53 (bd, 0.9 H, NH, $J_{NH,2}$ = 9.7), 5.20 (d, 1 H, 4-OH, $J_{OH,4}$ = 2.5), 4.87 (d, 1 H, H-1α, $J_{1,2}$ = 4.1), 4.78 (d, 1 H, 1-Bn, J_{gem} = 12.7), 4.62 (s, 2 H, 6-Bn and 6-Bn'), 4.46 (d, 1 H, 1-Bn'), 4.21 (m, 1 H, CH), 4.11 (ddd, 1 H, H-2, $J_{2,3}$ = 10.7), 3.88 (ddd, 1 H, H-5), 3.85 (dd, 1 H, H-6eq, J_{gem} = 10.7, $J_{5,6eq}$ = 2.0), 3.73 (dd, 1 H, H-6ax, $J_{5,6ax}$ = 5.6), 3.70 (s, 3 H, OMe), 3.62 (ddd, 1 H, H-4, $J_{4,5}$ = 9.7), 3.44 (dd, 1 H, H-3, $J_{3,4}$ = 8.6), 2.59 (dd, 1 H, CH_2ax, $J_{CH,ax}$ = 9.7, J_{gem} = 17.0), 2.43 (dd, 1 H, CH2eq, $J_{CH,eq}$ = 2.5), 1 94 (s, 3 H, Ac), 1.10 (d, 3 H, CH_3, $J_{CH,CH3}$ = 6.2).

Anal. Calcd for $C_{27}H_{35}NO_8$: C 64.66 H 7.03 N 2.79 Found: C 64.52 H 7.05 N 2.79

Benzyl 2-acetamido-6-O-benzyl-4-O-[2(R)-1-carboxymethyl-isopropyl]-a-D-glucopyranoside (**20**) and *Benzyl 2-acetamido-6-O-benzyl-4-O-[2(S)-1-carboxymethyl-isopropyl]-a-D-glucopyranoside* (**21**): A mixture of **17** and **18** (10 mg) was dissolved in dry methanol (10 mL), a saturated solution of hydrochloric acid in diethylether (1 mL) was added and the mixture stirred over night. The solvent was evaporated, the residue dissolved in dichloromethane (10 mL), washed with saturated sodium hydrogencarbonate solution,

dried ($MgSO_4$) and filtered. Evaporation of the solvent yielded a mixture of **20** and **21** (10 mg, 97%) – ^{1}H-NMR ($CDCl_3$): 7.40-7.25 (m, 10 H, Ph), 5.77 and 5.65 (d, 0.4 H, NH, $J_{NH,2}$ = 8.7), 5.06 (d, 0.4 H, 4-OH bridged, $J_{OH,4}$ = 3.0), 5.05 and 4.91 (d, 0.5 H, H-1α, $J_{1,2}$ = 4.1), 4.75-4.42 (m, 4 H, Bn), 4.31 and 4.21 (ddd, 0.5 H, CH), 4.18-4.10 (m, 1 H, H-2), 3.82-3.45 (m, 5 H, H-3, H-4, H-5, H6, H-6′), 3.70 and 3.64 (s, 1.5 H, OMe), 3.02 (d, 0.4 H, 4-OH free, $J_{OH,4}$ = 4 1), 2.56 and 2.32 (dd, 0 5 H, CH_2, J_{CH,CH_2} = 6.6, J_{gem} = 15.3), 2.50 (dd, 1 H, CH_2′), 1.98 (s, 3 H, Ac), 1.25 and 1.09 (d, 1.5 H, CH_3, $J_{CH,Me}$ = 6.1).



Numbering of protons for NMR data of **23** and **25** (non IUPAC)

Benzyl 2-acetamido-6-O-benzyl-3-O-[N-alanyl-allylester-2(S)-1-carbamido-isopropyl]-a-D-gluco-pyranoside (**23**): To a stirred solution of **22** (48 mg, 0.15 mmol) and triethylamine (21 μL, 0.15 mmol) in dichloromethane (1 mL) were added **15** (47 mg, 0.15 mmol) in dichloromethane (1 mL) and EEDQ (45 mg, 0.18 mmol). The reaction mixture was stirred over night at room temperature. Then it was diluted with dichloromethane (10 mL), washed successivly with 0.5 N hydrochloric acid, saturated sodium hydrocarbonate solution and water (3x 5 mL each) and dried ($MgSO_4$). Evaporation of the solvent and flash chromatography (toluene/ethanol 15:1) yielded **23** (foam, 59 mg, 65%); $[\alpha]_D^{20}$ +41 (*c* 3, chloroform) – ^{1}H-NMR ($CDCl_3$): δ = 7.40-7.20 (m, 10 H, Ph), 6.61 (d, 0.9 H, NH_{Ala}, $J_{NH,10}$ = 7.1), 5.94-5.82 (m, 1 H, H-c), 5.55 (d, 0.9 H, NH, $J_{NH,2}$ = 10.2), 5.43 (d, 1 H, 4-OH, $J_{OH,4}$ = 3.6), 5.37 -5.21 (m, 2 H, H-a and H-b), 4.86 (d, 1 H, H-1α, $J_{1,2}$ = 3.6), 4.75 (d, 1H, 1-Bn, J_{gem} = 11.7), 4.65-4.55 (m, 5 H, 6-Bn, 6-Bn′, H-d, H-d′, H-10), 4.46 (d 1 H, 1-Bn′), 4.29 (m, 1 H, H-7), 4.12 (ddd, 1 H, H-2, $J_{2,3}$ = 10.2), 3.87 (ddd, 1 H, H-5, $J_{4,5}$ = 8.7, $J_{5,6ax}$ = 5.6, $J_{5,6eq}$ = 2.0), 3.81 (dd, 1 H, H-6eq, J_{gem} = 10.7), 3.73 (dd, 1 H, H-6ax), 3,63 (ddd, 1 H, H-4, $J_{3,4}$ = 8.7), 3.45 (dd, 1 H, H-3), 2.43 (dd, 1 H, H-8eq, $J_{7,8eq}$ = 3.0, J_{gem} = 17.3), 2,37 (dd, 1 H, H-8ax, $J_{7,8ax}$ = 9.2), 1.95 (s, 3 H, Ac), 1.40 (d, 3 H, CH_3-11, $J_{10,11}$ = 7.1), 1.11 (d, 3 H, CH_3-9, $J_{7,9}$ = 6.1).

Anal. Calcd for $C_{32}H_{42}N_2O_9$: C 64.20 H 7.07 N 4.68 Found: C 64.01 H 7.09 N 4.66

Benzyl 2-acetamido-6-O-benzyl-3-O-[N-{L-alanyl-D-glutamic-acid-dimethylester}-2(S)-1-carb-amido-isopropyl]-a-D-glucopyranoside (**25**): To a stirred solution of **24** (22 mg, 0.09 mmol) in dichloromethane (1 mL) were added **15** (43 mg, 0.09 mmol) in dichloromethane (1 mL) and EEDQ (27 mg, 0.11 mmol). The reaction mixture was stirred over night at room temperature. Then it was diluted with dichloromethane (10 mL), washed successivly with 0.5 N hydrochloric acid, saturated sodium hydrocarbonate solution and water (3x 5 mL each) and dried ($MgSO_4$). Evaporation of the solvent and flash chromatography (dichloromethane/metanol 20:1) yielded **25** (foam, 33 mg, 52%); $[\alpha]_D^{20}$ +25 (*c* 0.6, chloroform) – ^{1}H-NMR ($CDCl_3$): δ = 7.40-7.20 (m, 10 H, Ph), 7.05 (d, 1 H, NH_{Glu}, $J_{NH,12}$ = 7.6), 6.99 (d, 1 H, NH_{Ala}, $J_{NH,10}$ = 7.1), 5.62 (d, 0.9 H, NH, $J_{NH,2}$ = 9.7), 5.42 (d, 0.9 H, 4-OH, $J_{4,OH}$ = 2.5), 4.86 (d, 1 H, H-1α, $J_{1,2}$ = 3.6), 4.74 (d, 1 H, 1-Bn, J_{gem} = 11.7), 4.68-4.43 (m, 4 H, 6-Bn, 6′-Bn, H-10, H-12), 4.45 (d, 1 H, 1-Bn), 4.24 (m, 1 H,

H-7), 4.14 (ddd, 1 H, H-2, $J_{2,3}$ = 10.2), 3.87 (ddd, 1 H, H-5, $J_{4,5}$ = 8.7, $J_{5,6ax}$ = 5.6, $J_{5,6eq}$ = 2.0), 3.83-3.58 (m, 3 H, H-6, H-6′, H-4), 3.70 (s, 3 H, OMe), 3.66 (s, 3 H, OMe), 3.49 (dd, 1 H, H-3, $J_{3,4}$ = 8.6), 2.50-1.85 (m, 6 H, CH_2-8, CH_2-13, CH_2-14), 1.94 (s, 3 H, Ac), 1.36 (d, 3 H, CH_3-11, $J_{10,11}$ = 6.6), 1.12 (d, 3 H, CH_3-9, $J_{7,9}$ = 6.1).

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