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Enzymatic synthesis of some *O*-β-D-digalactosyl glycopeptides, using β-D-galactosidase

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ABSTRACT

Disaccharide–peptide conjugates were obtained in yields of 30–50% from *o*-nitrophenyl β-D-galactopyranoside by employing β-D-galactosidase from *E. coli* as catalyst. Two series of β-D-galactosyldipeptides were examined as galactosyl acceptors. They both contain an L-serine residue β-linked to the anomeric carbon of galactose. In the first series, serine is in the N-terminal position of the dipeptide; in the second series, serine is in the C-terminal position. The second amino acid is L-alanine or glycine. Some of our substrates gave a high yield of β-(1 → 3)-digalactosyldipeptide derivatives and all gave very little of the β-(1 → 6) regioisomer. The conditions and the limitations of the transgalactosylation reaction are discussed.

INTRODUCTION

Even though chemical methods for the synthesis of glycosidic linkages are well developed[1–3], there is growing interest in new methods for one-step regio- and stereo-specific preparation of oligosaccharides and glycopeptides.

Glycosidases have long been known to catalyze not only the hydrolysis of glycosidic bonds but also the stereospecific formation of glycosidic linkages[4]. The syntheses have been achieved either by an equilibrium approach[4–7] or by a kinetic approach (transglycosylation)[8–11] using an appropriate glycoside donor.

As part of a programme to develop the enzymatic synthesis of glycopeptides, we showed previously that the stereospecific formation of a glycosidic bond between galactose (or glucose) and L-serine was possible using galactosidase (or glucosidase), as long as both the amino and carboxyl groups of serine were protected[12,13]. These results were confirmed recently by different groups[14–17], and Sauerbrei and Thiem[16] succeeded in the glycosylation of unprotected serine. We also showed that transglycosylations occurred with dipeptides bearing a serine residue[18].

In order to extend the enzymatic glycosidations to the synthesis of disaccharides containing serine (or peptides), we subjected *O*-β-D-galactosyl-L-serine to a further

transglycosylation reaction, using lactose as the galactosyl donor, and showed that the β-(1 → 3)- and β-(1 → 6)-digalactosylserine derivatives were obtained[18].

In this paper, we report on the formation of β-digalactosyldipeptides and a study of the conditions and the limitations of transglycosylations catalyzed by the β-D-galactosidase from *E. coli*.

RESULTS AND DISCUSSION

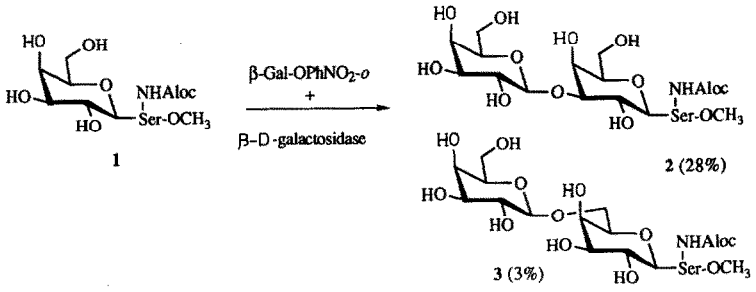
Previously, we chose lactose as the galactosyl donor for the condensation of the β-D-galactosylserine derivative **1** with β-D-galactosidase[18]. The β-(1 → 3)- and β-(1 → 6)-digalactosylserine derivatives **2** and **3** were obtained in 13 and 6% yield, respectively, as long as *N*,*N*-dimethylformamide was added to the mixture, Very poor yields were observed in the absence of *N*,*N*-dimethylformamide and also with other organic water-miscible solvents.

However, it is known that *o*-nitrophenyl β-D-galactopyranoside is a better donor than lactose for transglycosylation reactions[14–17]. Transgalactosylations with this donor were performed using β-D-galactosidase from *E. coli* and β-D-galactosylserine **1**. In contrast to what was observed with lactose as the galactosyl donor, *N*,*N*-dimethylformamide does not favor the formation of the expected β-digalactosylserine derivatives. Instead, *o*-nitrophenyl 6-*O*-β-D-galactopyranosyl-β-D-galactopyranoside and *o*-nitrophenyl 3-*O*-β-D-galactopyranosyl-β-D-galactopyranoside were obtained as the major products. Only small amounts of the desired digalactosylserine derivatives were observed. This was also the case if acetonitrile (or acetone) was used as cosolvent. These results are in agreement with those described by Sauerbrei and Thiem[16] in their synthesis of nitrophenyl disaccharide-glycosides. However, with no organic cosolvent added and with high concentrations of the reactants, we now show that condensations take place and sometimes with fair yield.

In these β-D-galactosidase-catalyzed transglycosylations, serine is protected by an allyloxycarbonyl group on the amine and by an ester group on the acid. In comparison with lactose, the reactions with *o*-nitrophenyl β-D-galactopyranoside gave a higher yield (28%) of the β-(1 → 3)-digalactosylserine **2** (as compared to 13%) and a smaller amount (< 3%) of the β-(1 → 6) regioisomer **3** (as compared to 6%) (Scheme 1).

This type of transfer was also performed on two series of *O*-β-D-galactosyldipeptide derivatives (**4–7**, **14**, and **15**, Scheme 2). Both series contain a serine residue β-linked to the anomeric carbon of D-galactose. In the first series, serine is in the N-terminal position of the dipeptide; in the second series, serine is in the C-terminal position. The second amino acid is alanine or glycine.

It appears that the transglycosylations work well with some galactosyldipeptides of the first series since with (*N*-allyloxycarbonyl-3-*O*-β-D-galactopyranosyl-L-seryl)-L-alanine methyl ester (**4**), for example, condensation with *o*-nitrophenyl β-D-galactopyranoside occurred with a total yield of 52% for the two expected



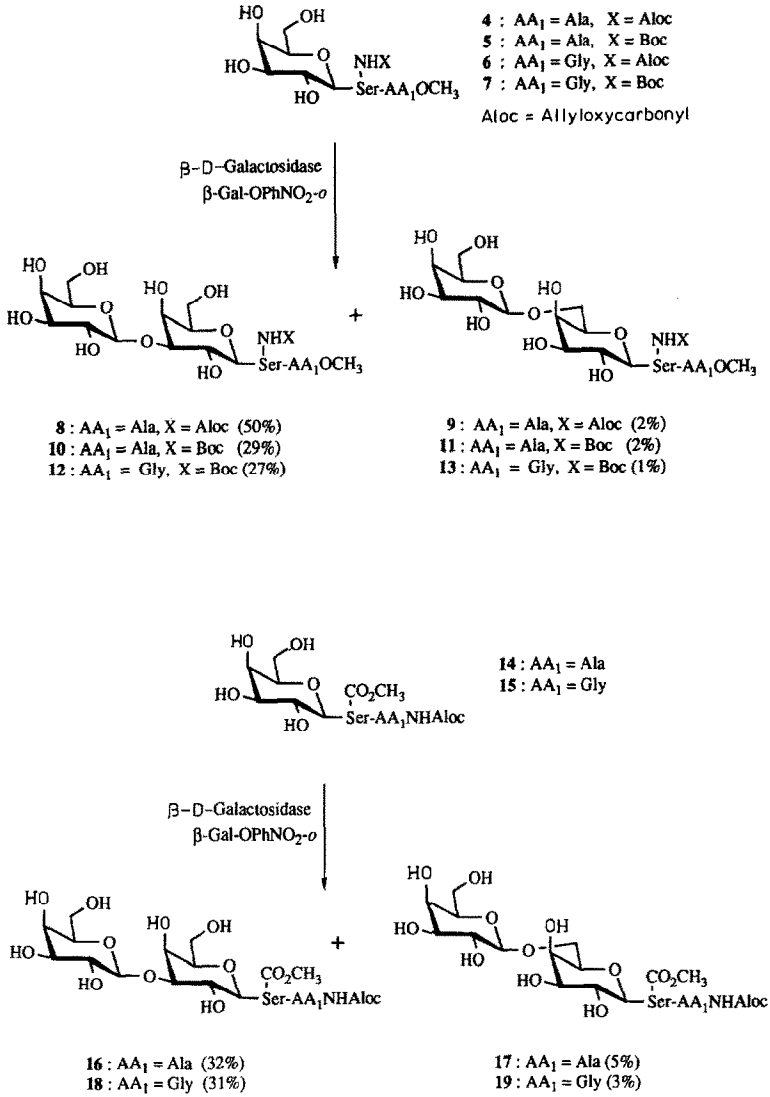
Scheme 1.

digalactosyldipeptide regioisomers. As observed before, the β-(1 → 3) linkage between the two galactosyl residues was formed preferentially [50% yield of **8** after purification and only 2% of the β-(1 → 6) regioisomer **9**]. Surprisingly, if glycine is the second amino acid (compound **6**), condensation does not take place. We observed a very poor solubility of this galactosyldipeptide derivative, and we think it is the reason why the transglycosylation does not occur under our conditions. Higher dilution gives mainly the *o*-nitrophenyl β-digalactoside derivatives.

In a previous paper, we showed that condensations of dipeptide derivatives with lactose as the donor were influenced by the position of the serine residue in the dipeptide but not by the nature of the amine protective group (*tert*-butoxycarbonyl or allyloxycarbonyl)[18]. So we submitted the β-D-galactosyldipeptide **7** (protected by a *tert*-butoxycarbonyl group on the amine and, apparently, more soluble in water) to the transgalactosylation reaction. In fact condensation is observed but the yield of the expected β-(1 → 3)-digalactosyldipeptide **12** is lower than for the digalactosylserylalanine derivative **8** protected by an allyloxycarbonyl group (27% as compared to 50% for **8**). Furthermore, a higher quantity of enzyme is necessary to attain this yield and the time of incubation is greater. This lower reactivity of galactosyldipeptide **7**, as compared to **4**, is unexpected since the condensations of lactose with serylalanine or serylglycine dipeptides gave similar results with both the amine protective groups (Boc or Aloc)[18].

The influence of the *tert*-butoxycarbonyl group as amine protective group was further studied in the reaction of *o*-nitrophenyl β-D-galactopyranoside with the galactosylserylalanine derivative **5**. In fact, the yield of the expected β-(1 → 3)-digalactosyldipeptide **10** was 29% as compared to 50% for the homologous derivative **8** protected by an allyloxycarbonyl group. These results show a clear influence of the amine protecting group, an influence which had not been observed in the reaction of lactose with dipeptide ester derivatives.

For the galactosyldipeptides of the second series (**14** and **15**), the nature of the amino acid AA_1 (alanine or glycine) did not influence the yield, which was somewhat lower than for the derivative **8** of the first series [31% of the β-(1 → 3)-digalactosyldipeptides **16** and **18** as compared to 50% for **8**]. Again the β-(1 → 6)



Scheme 2.

regioisomers **17** and **19** were formed in a very poor yield (5 and 3%, respectively). Here the two substrates **14** and **15** gave the same results, as expected (glycylserine and alanylserine dipeptides behaved similarly in their reaction with lactose).

In all these transgalactosylations, we used a five-fold excess of the acceptor to minimize the amount of *o*-nitrophenyl 3-*O*- and 6-*O*-β-D-galactopyranosyl-β-D-galactopyranoside formed in a competitive reaction. However, after purification on silica gel, small amounts of these derivatives were still present (5–8%) and further purification by HPLC was necessary to give the analytically pure compounds.

This method of synthesis of β-digalactosyldipeptide derivatives is very attractive since it is stereospecific and gives mainly the β-(1 → 3) linkage in good yields (50% for some of our substrates). Only traces of the β-(1 → 6) regioisomers are observed. The reactions are usually fast (3 h) and easy to follow by TLC (disappearance of *o*-nitrophenyl β-D-galactopyranoside, UV light).

Some examples of enzymatic galactosylation of glycopeptides are described in the literature[19–22]. The authors used a galactosyltransferase with UDP-galactose as the glycosyl donor. These transferases catalyzed condensations with *N*-acetylglucosamine–peptide conjugates as the acceptor to give β-(1 → 4)-linked disaccharide–peptide derivatives.

This paper shows that β-D-galactosidase from *E. coli* catalyzed another type of transfer from an easily accessible galactosyl donor (*o*-nitrophenyl β-D-galactopyranoside) to give β-digalactosylpeptide conjugates with a β-(1 → 3) linkage between the two galactose residues.

EXPERIMENTAL

General.—β-D-Galactosidase (*E. coli*) was obtained from Boehringer (100 U/mg of lyophilisate). A solution of β-D-galactosidase was prepared by dissolving the enzyme powder (1 mg) in 1 mL of 0.03 M sodium phosphate buffer (pH 7.8) that contained mM $MgCl_2$ and 5 mM dithiothreitol. The *tert*-butoxycarbonyldipeptide esters were prepared by the classical method with dicyclohexylcarbodiimide and 1-hydroxybenzotriazole[23]. The allyloxycarbonyl group was introduced on the dipeptide ester as described in the literature for the *tert*-butoxycarbonyl group[24]. β-Galactosyldipeptide derivatives were synthesized either chemically (**4**, **6**, **14**, and **15**) or enzymatically (**5** and **7**). The chemical method was a condensation between β-D-galactose pentaacetate and the appropriate allyloxycarbonyldipeptide ester, using trimethylsilyl trifluoromethanesulfonate as catalyst[25], followed by deacetylation[26]; the overall yield was 30%. In the enzymatic syntheses of **5** and **7** (see below), galactosyl tranfer was monitored by TLC on Silica Gel F_{254} (Merck) with CH_2Cl_2–MeOH (20%) or 7:1:2 2-propanol–aq 20% NH_3–water, and detection was made by spraying 3,5-dihydroxytoluene (0.5% in 10 M H_2SO_4). HPLC was performed using a Perkin–Elmer pump system with a light-diffusion monitor (Touzart et Matignon, France). A column (150 × 4.6 mm) of Nucleosil C_{18} (5 μm) was used and the products were eluted with an acetonitrile–water gradient (5–13% for **8** and **16**, 0.5–20% for **12** and **18**). Optical rotations were measured at 589 nm (sodium line) on a Perkin–Elmer 241 MC polarimeter. ^1H NMR spectra [300 MHz, internal 3-(trimethylsilyl)propionic acid, sodium salt] were recorded with a Bruker instrument. Chemical shifts for ^{13}C NMR data are given relative to that for 1,4-dioxane (67.86 ppm downfield from the signal for Me_4Si). Mass spectra were recorded on a VG 70-250 double focusing instrument (VG instruments, Le Chesnay, France) equipped with a fast atom bombardment gun (Ion Tech., UK). The gun was operated with Xe at 8 kv and 1 mA.

After purification on silica gel, the products were always contaminated by 5 to 8% of the *o*-nitrophenyl β-digalactosides and HPLC was necessary to obtain the analytically pure samples. Elemental analyses are given for some representative compounds (**8, 12, 16,** and **18**). The β-(1 → 6) regioisomers were present in very small quantities and could not therefore be fully characterized. However, they all showed a ^{13}C NMR signal around 69.6 ppm, typical of a C-6 carbon linked to C-1 of galactose[27–29].

*(*N-tert-*Butoxycarbonyl-3-*O-β-D-*galactopyranosyl-*L-*seryl)glycine methyl ester* (**7**).—This derivative has been prepared before[18] using lactose as galactosyl donor. The procedure described here used *o*-nitrophenyl β-D-galactopyranoside as donor since the reaction is faster and easier to follow by TLC. The yields were similar to those obtained from lactose. *o*-Nitrophenyl β-D-galactopyranoside (812 mg, 2.7 mmol) was incubated with BocSerGlyOMe (2.5 g, 9 mmol) in 2 mL of phosphate buffer and 3.5 mL (350 U) of β-D-galactosidase. The end of the reaction (disappearance of the donor) was followed by TLC (4:1 CH_2Cl_2–MeOH). The expected derivative **7** was obtained as described before (123 mg, 14%).

*(*N-tert-*Butoxycarbonyl-3-*O-β-D-*galactopyranosyl-*L-*seryl)-*L-alanine methyl ester* (**5**).—The same procedure as for **7** above was used, and compound **5** was obtained in 11% yield.

N-*Allyloxycarbonyl-3-*O-*(3-*O- *and 6-*O-β-D-*galactopyranosyl-β-D-galactopyranosyl)-*L-serine methyl ester (***2** *and* **3**).—*o*-Nitrophenyl β-D-galactopyranoside (60 mg, 0.2 mmol) was incubated with 365 mg (1 mmol) of the galactosylserine derivative **1** in 560 μL of the buffered solution of β-D-galactosidase (56 U). After the end of the reaction (3 h), the mixture was processed as described before[18]: 29 mg (28%) of the expected β-(1 → 3)-digalactosylserine derivative **2** was obtained as well as 3 mg (3%) of the β-(1 → 6) regioisomer **3** (data give in a previous paper[18]).

*[*N-*Allyloxycarbonyl-3-*O-*(3-*O- *and 6-*O-β-D-*galactopyranosyl-β-D-galactopyranosyl)-*L-seryl]-*L-alanine methyl ester (***8** *and* **9**).—*o*-Nitrophenyl β-D-galactopyranoside (60 mg, 0.2 mmol) was incubated at room temperature with 436 mg (1 mmol) of the galactosyserylalanine derivative **4** in 540 μL of phosphate buffer and 280 μL of a buffered solution of β-D-galactosidase (28 U). After complete disappearance of the donor (3 h), the mixture was adsorbed on silica gel and subjected to column chromatography on silica gel (Merck, 0.040–0.063 mm). Elution was realized by using a gradient of CH_2Cl_2 with 60:35:10.8 CH_2Cl_2–MeOH–EtOH–H_2O: compound **8** (60 mg, 50%) was obtained first and then **9** (2.5 mg, 2%).

Compound **8**: mp 165°C; $[\alpha]_D$ −9.6° (*c* 1.4, H_2O); NMR data (D_2O): ^1H, δ 1.42 (d, *J* 7.3 Hz, CH_3 Ala), 3.61–3.78 (H-5, H-5′, H-6, H-6′, $COOCH_3$), 3.58 (H-2′), 3.63 (H-3′), 3.68 (H-2), 3.82 (H-3), 3.9 (H-4′), 3.95–4.41 (CH_2 Ser), 4.18 (H-4), 4.15 (CHα Ser), 4.45 (CHα Ala, H-1), 4.6 (H-1′, *J* 6.9 Hz, CH_2 allyl), 5.3 (CH_2 allyl), 5.95 (CH=); ^{13}C, δ 175.43 (CONH), 172.55 (COOMe), 158.5 (OCONH), 133.16 (CH=), 118.1 (CH_2=), 104.97–103.12 (C-1, C-1′), 82.81 (C-3), 75.72–75.51 (C-5, C-5′), 73.17 (C-3′), 71.70–70.50 (C-2, C-2′), 69.35 (CH_2 Ser), 69.22–69.02 (C-4, C-4′), 66.89 (CH_2 allyl), 61.61 (C-6, C-6′), 55.56 (CHα Ser), 53.62 (CO_2CH_3),

49.47 (CHα Ala), 16.66 (CH_3 Ala). Mass spectrum, FAB: m/z 599 ($M + H^+$). Anal. Calcd for $C_{23}H_{38}N_2O_{16} \cdot 1.5H_2O$: C, 44.16; H, 6.60; N, 4.48; O, 44.75. Found: C, 43.99; H, 6.51; N, 4.51; O, 45.02.

*[*N-tert-*Butoxycarbonyl-3*-O-*(3*-O- *and 6*-O-*β*-D-*galactopyranosyl-β*-D-*galactopyranosyl)*-L-*seryl]*-L-*alanine methyl ester (***10** *and* **11**).—The same conditions as for the synthesis of compounds **8** and **9** were used. From 60 mg (0.2 mmol) of *o*-nitrophenyl β-D-galactopyranoside, 36 mg (29%) of compound **10** was obtained. Only traces of the β-(1 → 6) derivative **11** were observed (2 mg, 2%).

Compound **10**: mp 117°C; $[\alpha]_D$ −13.3° (*c* 0.9, H_2O); NMR data (D_2O): ^1H, δ 1.41 (CH_3 Ala), 1.42 (Boc), 3.61–3.77 (H-5, H-5′, H-6, H-6′, $COOCH_3$), 3.58 (H-2′), 3.64 (H-3′), 3.69 (H-2), 3.8 (H-3), 3.9 (H-4′), 3.9-4.14 (CH_2 Ser), 4.18 (H-4), 4.3 (CHα Ser), 4.45 (d, *J* 7.6 Hz, H-1; CHα Ala), 4.59 (d, *J* 7.3 Hz, H-1′); ^{13}C, δ 175.65 (CONH), 173.05 (COOMe), 158.26 (OCONH), 105.08–103.18 (C-1, C-1′), 82.92 (C-3), 75.83–75.62 (C-5, C-5′), 73.27 (C-3′), 71.79–70.60 (C-2, C-2′), 69.45 (CH_2 Ser), 69.33–69.13 (C-4, C-4′), 61.72 (C-6, C-6′), 55.42 (CHα Ser), 53.71 (CO_2CH_3), 49.55 (CH Ala), 28.31 [$C(CH_3)_3$]. Mass spectrum, FAB: m/z 615 ($M + H^+$).

*[*N-tert-*Butoxycarbonyl-3*-O-*(3*-O- *and 6*-O-*β*-D-*galactopyranosyl-β*-D-*galactopyranosyl)*-L-*seryl]glycine methyl ester* (**12** *and* **13**).—*o*-Nitrophenyl β-D-galactopyranoside (60 mg, 0.2 mmol) was incubated with the galactosylserylglycine derivative **7** (438 mg, 1 mmol) in 270 μL of buffer and a solution of β-D-galactosidase in the buffer (600 μL, 60 U). After disappearance of the donor (6 h), the mixture was processed as described above and 32 mg (27%) of compound **12** was obtained as well as traces of **13** (1 mg, 1%).

Compound **12**: mp 98°C; $[\alpha]_D$ +5.5° (*c* 1, H_2O); NMR data (D_2O): ^1H, δ 1.41 (Boc), 3.62–3.9 (H-5, H-5′, H-6, H-6′, $COOCH_3$), 3.58 (H-2′), 3.65 (H-3′), 3.68 (H-2), 3.8 (H-3), 3.9 (H-4′), 3.9–4.2 (CH_2 Ser), 4.05 (CH_2 gly), 4.18 (H-4), 4.35 (CHα Ser), 4.45 (d, *J* 7.8 Hz, H-1), 4.6 (d, *J* 7.3 Hz, H-1′); ^{13}C, δ 173.72 (CONH), 172.50 (COOMe), 158.18 (OCONH), 105.02–103.19 (C-1, C-1′), 82.85 (C-3), 75.77–75.55 (C-5, C-5′), 73.22 (C-3′), 71.74–70.54 (C-2, C-2′), 69.51 (CH_2 Ser), 69.27–69.07 (C-4, C-4′), 61.65 (C-6, C-6′), 55.45 (CHα Ser), 53.51 (CO_2CH_3), 41.95 (CH_2Gly), 28.26 [$C(CH_3)_3$]. Mass spectrum, FAB: m/z 601 ($M + H^+$). Anal. Calcd for $C_{23}H_{40}N_2O_{16} \cdot 1.5H_2O$: C, 44.02; H, 6.91; N, 4.46; O, 44.61. Found: C, 44.31; H, 6.82; N, 4.46; O, 45.08.

*(*N-*Allyloxycarbonyl*-L-*alanyl)-3*-O-*(3*-O- *and 6*-O-*β*-D-*galactopyranosyl-β*-D-*galactopyranosyl)*-L-*serine methyl ester (***16** *and* **17**).—The conditions used for the condensation of the galactosyldipeptide **14** were the same as for the homologous derivative **4** (see above). From 60 mg (0.2 mmol) of *o*-nitrophenyl β-D-galactopyranoside, 38 mg (32%) of the β-(1 → 3)-digalactosyldipeptide **16** as well as 6 mg (5%) of the β-(1 → 6) regioisomer **17** were obtained after purification.

Compound **16**: mp 109°C; $[\alpha]_D$ −1.5° (*c* 1.3, H_2O); NMR data (D_2O): ^1H, δ 1.36 (d, *J* 7.3 Hz, CH_3 Ala), 3.61–3.78 (H-5, H-5′, H-6, H-6′, $COOCH_3$), 3.58 (H-2′), 3.67 (H-2), 3.8 (H-3), 3.9 (H-4′), 3.95–4.35 (CH_2 Ser), 4.18 (CHα Ala, H-4),

4.4 (d, *J* 7.7 Hz, H-1), 4.56 (CH_2 allyl, H-1′), 4.73 (CHα Ser), 5.3 (CH_2 allyl), 5.95 (CH=); ^{13}C, δ 176.9 (CONH), 172.54 (COOMe), 158.5 (OCONH), 133.46 (CH=), 118.15 (CH_2=), 105.18–103.57 (C-1, C-1′), 83.05 (C-3), 75.93–75.67 (C-5, C-5′), 73.37 (C-3′), 71.89–70.68 (C-2, C-2′), 69.53 (CH_2 Ser), 69.42–69.18 (C-4, C-4′), 66.85 (CH_2 allyl), 61.8–61.7 (C-6, C-6′), 54.07 ($COOCH_3$), 53.74 (CHα Ser), 51.55 (CHα Ala), 17.69 (CH_3 Ala). Mass spectrum, FAB: *m/z* 599 ($M + H^+$). Anal. Calcd for $C_{23}H_{38}N_2O_{16} \cdot 1.5H_2O$: C, 44.16; H, 6.60; N, 4.48; O, 44.75. Found: C, 44.31; H, 6.52; N, 4.46; O, 45.18.

Compound **17**: NMR data (D_2O): ^{13}C, δ 176.87 (CONH), 172.49 (COOMe), 158.5 (OCONH), 133.44 (CH=), 118.16 (CH_2=), 104.15–103.89 (C-1, C-1′), 76.00–74.69 (C-5, C-5′), 73.54–73.29 (C-3, C-3′), 71.56–71.46 (C-2, C-2′), 69.69 (CH_2 Ser, C-6), 69.45 (C-4, C-4′), 66.85 (CH_2 allyl), 61.82 (C-6′), 54.07 ($COOCH_3$), 53.73 (CHα Ser), 51.54 (CHα Ala), 17.69 (CH_3 Ala). Mass spectrum, FAB: *m/z* 599 ($M + H^+$).

*(*N*-Allyloxycarbonylglycyl)-3-*O*-(3-*O*- and 6-*O*-β-*D*-galactopyranosyl-β-*D*-galactopyranosyl)-*L*-serine methyl ester (***18*** and ***19***).*—The conditions used for the condensation of the galactosyldipeptide **15** were the same as for **14** above. From 60 mg (0.2 mmol) of *o*-nitrophenyl β-D-galactopyranoside, 36 mg (31%) of the digalactosyldipeptide **18** was obtained after purification, as well as 3 mg (3%) of the regioisomer **19**.

Compound **18**: mp 108°C; $[\alpha]_D$ +7.9° (*c* 1.9, water); NMR data (D_2O): ^1H, δ 3.66–3.81 (H-5, H-5′, H-6, H-6′, $COOCH_3$), 3.6 (H-2′), 3.68 (H-2), 3.8 (H-3), 3.9 (CH_2 Gly, H-4′), 3.97–4.35 (CH_2 Ser), 4.18 (H-4), 4.44 (d, *J* 7.7 Hz, H-1), 4.6 (CH_2 allyl, H-1′), 4.78 (CHα Ser), 5.3 (CH_2 allyl), 5.95 (CH=); ^{13}C, δ 173.18 (CONH), 172.54 (COOMe), 159.34 (OCONH), 133.36 (CH=), 118.38 (CH_2=), 105.13–103.53 (C-1, C-1′), 82.96 (C-3), 75.89–75.64 (C-5, C-5′), 73.33 (C-3′), 71.85–70.61 (C-2, C-2′), 69.52 (CH_2 Ser), 69.38–69.15 (C-4, C-4′), 67.08 (CH_2 allyl), 61.76–61.67 (C-6, C-6′), 54.06 ($COOCH_3$), 53.70 (CHα Ser), 44.21 (CH_2 Gly), 17.69 (CH_3 Ala). Mass spectrum, FAB: *m/z* 585 ($M + H^+$). Anal. Calcd for $C_{22}H_{36}N_2O_{16} \cdot 1.5H_2O$: C, 43.21; H, 6.42; N, 4.58; O, 45.78. Found: C, 43.31; H, 6.52; N, 4.46; O, 45.18.

REFERENCES

1 R.R. Schmidt, *Angew. Chem. Int. Ed. Engl.*, 25 (1986) 212–235.
2 H.G. Garg and R.W. Jeanloz, *Adv. Carbohydr. Chem. Biochem.*, 43 (1985) 135–201.
3 H. Kunz, *Angew. Chem. Int. Ed. Engl.*, 26 (1987) 294–308.
4 K. Wallenfels and R. Weil, in P.D. Boyer (Ed.), *The Enzymes*, 3rd ed., Vol. 7, Academic, New York, 1972, pp 617–663.
5 R.A. Dedonder, *Annu. Rev. Biochem.*, 30 (1961) 347–382.
6 E. Johansson, L. Hedbys, P. Larsson, K. Mosbach, A. Gunnarsson, and S. Svensson, *Biotechnol. Lett.*, 8 (1986) 421–424.
7 K. Wallenfels, *Bull. Soc. Chim. Biol.*, 42 (1960) 1715.
8 L. Hedbys, P. Larsson, K. Mosbach, and S. Svensson, *Biochem. Biophys. Res. Commun.*, 123 (1984) 8–15.

9 K.G.I. Nilsson, *Carbohydr. Res.*, 167 (1987) 95–103.
10 K.G.I. Nilsson, *Carbohydr. Res.*, 180 (1988) 53–59.
11 A. Alessandrini, E. Schmidt, E. Zilliken, and F. György, *J. Biol. Chem.*, 220 (1956) 71–78.
12 D. Cantacuzène and S. Attal, *Carbohydr. Res.*, 211 (1991) 327–331.
13 D. Cantacuzène, S. Attal, and S. Bay, *Biomed. Biochim. Acta*, 50 (1991) 231–236.
14 N.J. Turner and M.C. Webberley, *J. Chem. Soc., Chem. Commun.*, (1991) 1349–1350.
15 E. Johansson, L. Hedbys, and P.O. Larsson, *Enzyme Microb. Technol.*, 13 (1991) 781–787.
16 B. Sauerbrei and J. Thiem, *Tetrahedron Lett.*, 33 (1992) 201–204.
17 E.W. Holla, M. Schudok, A. Weber, and M. Zulauf, *J. Carbohydr. Chem.*, 11 (1992) 659–663.
18 S. Attal, S. Bay, and D. Cantacuzène, *Tetrahedron*, 48 (1992) 9251–9260.
19 C. Augé, C. Gautheron, and H. Pora, *Carbohydr. Res.*, 193 (1989) 288–293.
20 J. Thiem and T. Wiemann, *Angew. Chem. Int. Ed. Engl.*, 29 (1990) 80–82.
21 J. Thiem and T. Wiemann, *Synthesis*, (1992) 141–145.
22 M. Schultz and H. Kunz, *Tetrahedron Lett.*, 33 (1992) 5319–5322.
23 W. König and R. Geiger, *Chem. Ber.*, 103 (1970) 788.
24 T. Brown, J.H. Jones, and J.D. Richards, *J. Chem. Soc., Perkin Trans. 1*, (1982) 1553–1561.
25 H. Paulsen and M. Brenken, *Liebigs Ann. Chem.*, (1988) 649–654.
26 V.A. Derevitskaya, M.G. Vagina, and N.K. Kochetkov, *Carbohydr. Res.*, 3 (1967) 377–388.
27 V.K. Srivastava, S.J. Sondheimer, and C. Schuerch, *Carbohydr. Res.*, 86 (1980) 203–214.
28 S.A. Abbas, J.J. Barlow, and K.L. Matta, *Carbohydr. Res.*, 98 (1981) 37–49.
29 E. Falentert-Kwast, P. Kovac, A. Bax, and C.P.J. Glaudemans, *Carbohydr. Res.*, 145 (1986) 332–340.