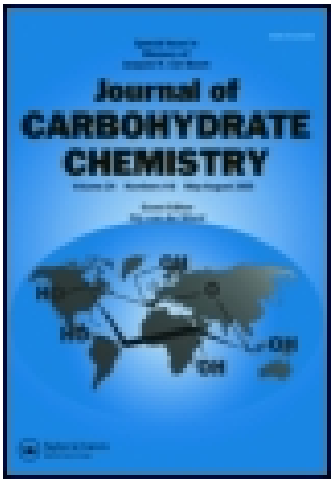


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# Mimics of the Structural Elements of Type III Group B Streptococcus Capsular Polysaccharide. Part I: Synthesis of a Carboxylate-Containing Pentasaccharide

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# MIMICS OF THE STRUCTURAL ELEMENTS OF TYPE III GROUP B *STREPTOCOCCUS* CAPSULAR POLYSACCHARIDE. PART I: SYNTHESIS OF A CARBOXYLATE-CONTAINING PENTASACCHARIDE

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## ABSTRACT

A carboxylate-containing pentasaccharide, methyl *O*-(β-D-galactopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyl)-(1→6)-*O*-{3-*O*-[(*S*)-1-carboxyethyl]-β-D-galactopyranosyl-(1→4)-*O*}-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside (**27**) was synthesized by block condensation of suitably protected donors and acceptors. Phenyl 3-*O*-benzyl-4,6-di-*O*-chloroacetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**17**) was condensed with methyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**4**) to afford a disaccharide, methyl *O*-(3-*O*-benzyl-4,6-di-*O*-chloroacetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**18**). Removal of chloroacetyl groups gave 4,6-diol, methyl *O*-(3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**19**), in which the primary hydroxy group (6-OH) was then selectively chloroacetylated to give methyl *O*-(3-*O*-benzyl-6-*O*-chloroacetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**20**). This acceptor was then coupled with 2,4,6-tri-*O*-acetyl-3-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-α-D-galactopyranosyl trichloroacetimidate (**14**) to afford a trisaccharide, methyl *O*-{2,4,6-tri-*O*-acetyl-3-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-β-D-galactopyranosyl}-(1→4)-*O*-(3-*O*-benzyl-6-*O*-chloroacetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**21**). Removal of the 6-*O*-

chloroacetyl group in **21** gave **22**, which was coupled with 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**23**) to yield protected pentasaccharide **24**. Standard procedures were used to remove acetyl groups and the phthalimido group, followed by *N*-acetylation, and debenzylation to yield pentasaccharide **27** and a hydrazide by-product (**28**) in a 5:1 ratio, respectively. Compound **27** contains a complete repeating unit of the capsular polysaccharide of type III group B *Streptococcus* in which terminal sialic acid is replaced by an (*S*)-1-carboxyethyl group.

## INTRODUCTION

Group B *Streptococcus* (GBS) is a major cause of neonatal sepsis and meningitis, and type III GBS causes more than 60% of all group B streptococcal infections.[1,2] The importance of capsular polysaccharide-specific antibodies in immunity to GBS III infection has been established,[3,4] and type III polysaccharide-based vaccines have been used in human clinical trials.[5] As part of our program to develop improved vaccines against group B streptococcal infections in humans,[6] we have been investigating the structural factors governing the binding of GBS III polysaccharide to its homologous antibodies. This interaction could not be inhibited by either of two overlapping pentasaccharide repeating units of GBS III polysaccharide prepared by enzymatic degradation[7] or by synthesis,[8] which can be interpreted as the failure of even those large oligosaccharide fragments to assume a particular conformation inherent to the GBS III polysaccharide.[9]

The fact that neither carboxyl-reduced nor desialylated GBS III polysaccharide bound to homologous antibody also revealed that although non-immunogenic,[10-12] sialic acid must play an important role in the formation of the conformational epitope.[9] Furthermore, the conformation of this epitope is not even dependent on the presence of intact sialic acid, as the partially oxidized GBS III polysaccharide in which both C-8 and C-9 had been removed from the exocyclic chain of sialic acid residues, still bound to homologous antibodies.[12] This led to the hypothesis that the epitope could conceivably be controlled by the carboxylate groups of sialic acid residues.

To further investigate this hypothesis it was decided to synthesize oligosaccharide probes in which sialic acid was replaced by surrogate anionic groups. Such an approach has been demonstrated in the successful binding of sialyl-Le$^a$ and -Le$^x$ to E-selectin when sialic

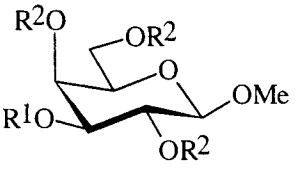
acid was replaced by a sulphate group.[13,14] For this study we chose the lactic acid-ether substituent[15] as the surrogate, which when introduced at the 3-*O*-position of the terminal β-D-galactopyranosyl residue, puts a carboxylate group in the same position as it is in the GBS III polysaccharide.

Here we describe the synthesis of a basal carboxylate-containing pentasaccharide **27** that contains the structural elements of GBS III capsular polysaccharide. Other related syntheses of various oligosaccharide fragments of the native[8,16,17] and desialylated native polysaccharides,[18-20] which has the same structure as the type 14 pneumococcal polysaccharide, have been reported.

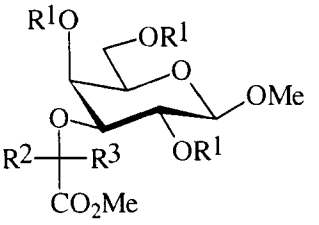


## RESULTS AND DISCUSSION

Methyl 3-*O*-allyl-β-D-galactopyranoside (**2**)[21-23] was fully benzylated (NaH/DMF/BnBr) to afford methyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**3**)[21] in 86% yield. The allyl group of **3** was removed by treatment with palladium chloride in methanol[23,24] at room temperature to yield crystalline **4**, [22] in 88% yield. Treatment of compound **4** in DMF with NaH and methyl (*S*)-2-chloropropionate gave two products **5a** (57%) and **5b** (8%). On previous evidence, one might predict that an $S_N2$ reaction would occur that would result in the inversion of configuration at the asymmetric center,[25-27] thus producing the (*R*)-diastereomer as the major product. However, the optical rotation of **5a**

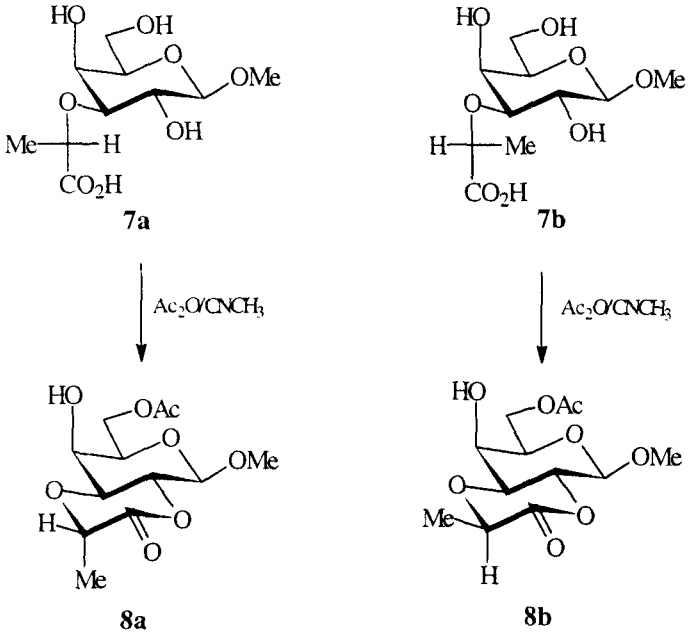



**1** $R^1$=$R^2$=H
**2** $R^1$=All $R^2$=H
**3** $R^1$=All $R^2$=Bn
**4** $R^1$=H $R^2$=Bn



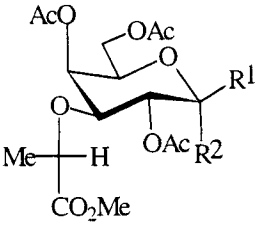

**5a** $R^1$=Bn $R^2$=Me $R^3$=H
**5b** $R^1$=Bn $R^2$=H $R^3$=Me
**6a** $R^1$=H $R^2$=Me $R^3$=H
**6b** $R^1$=H $R^2$=H $R^3$=Me

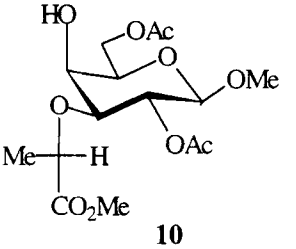
was more negative (-27°) than that of **5b** (-7°), which contradicted the prior observations with the similar reactions.[27] To unambiguously determine the absolute configuration of the 1-(methoxycarbonyl)ethyl group in **5a** and **5b**, the stereochemistry involved was investigated, as described by Severn and Richards[27] based on the formation of acetylated lactone derivatives **8a** and **8b**.




Conventional debenzylation of **5a** and **5b** ($H_2$/Pd-C) furnished **6a** and **6b**, respectively, in almost quantitative yield. Each of **6a** and **6b** were treated with 0.1 N NaOH at 80 °C for 5 h to yield **7a** and **7b**, which were then treated separately with acetic anhydride in acetonitrile to afford their respective diastereomeric lactone derivatives (**8a** and **8b**). Under these conditions the 6-*O*-position in both **8a** and **8b** was also acetylated. The relatively rigid 1,4-dioxane-2-one ring system in **8a** and **8b** permitted stereochemical assignments to be made by an NOE-based method.[27-29] The occurrence of specific NOEs between protons of the lactone and galactose ring systems indicated the orientation of the lactyl methyl group and α-H in **8a** and **8b**, and thus enabled assignments of the respective (*R*)- and (*S*)-lactones. For **8a** an NOE was observed between the lactyl methyl protons and

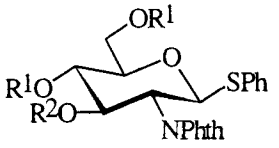
H-3 of galactose, while none was observed between the α-H and H-3 (see Figure 1), which indicated that **5a**, from which **8a** was obtained, was methyl 2,4,6-tri-*O*-benzyl-3-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-β-D-galactopyranoside. On the other hand, a strong NOE between lactone α-H and H-3 of galactose in **8b** (see Figure 2) indicated that **5b** was methyl 2,4,6-tri-*O*-benzyl-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-β-D-galactopyranoside.





**10**

**9** $R^1$=OMe $R^2$=H
**11** $R^1$=H $R^2$=Cl
**12** $R^1$=OAc $R^2$=H
**13** $R^1$,$R^2$=H, OH
**14** $R^1$=H $R^2$=OC(=NH)$CCl_3$



**15** $R^1$=$R^2$=Ac
**16** $R^1$=H $R^2$=Bn
**17** $R^1$=ClAc $R^2$=Bn

Compound **6a** was acetylated ($Ac_2O$/Pyr) at room temperature for 12 h to afford methyl 2,4,6-tri-*O*-acetyl-3-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-β-D-galactopyranoside (**9**) in 99% yield. It should be noted that a shorter reaction time (2 h at room temperature) gave exclusively methyl 2,6-di-*O*-acetyl-3-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-β-D-galactopyranoside (**10**). 2,4,6-Tri-*O*-acetyl-3-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-α-D-galactopyranosyl chloride (**11**) was prepared in 95% yield from **9** by reaction with 1,1-dichloromethyl methyl ether and a catalytic amount of zinc chloride.[30] In the presence of silver triflate and 2,6-lutidine,[23] **11** reacted with acetic acid giving 1,2,4,6-tetra-*O*-acetyl-3-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-β-D-galactopyranoside (**12**) in 85% yield, from which the 1-*O*-acetyl group was removed by reaction with hydrazine acetate in DMF,[31] affording 2,4,6-

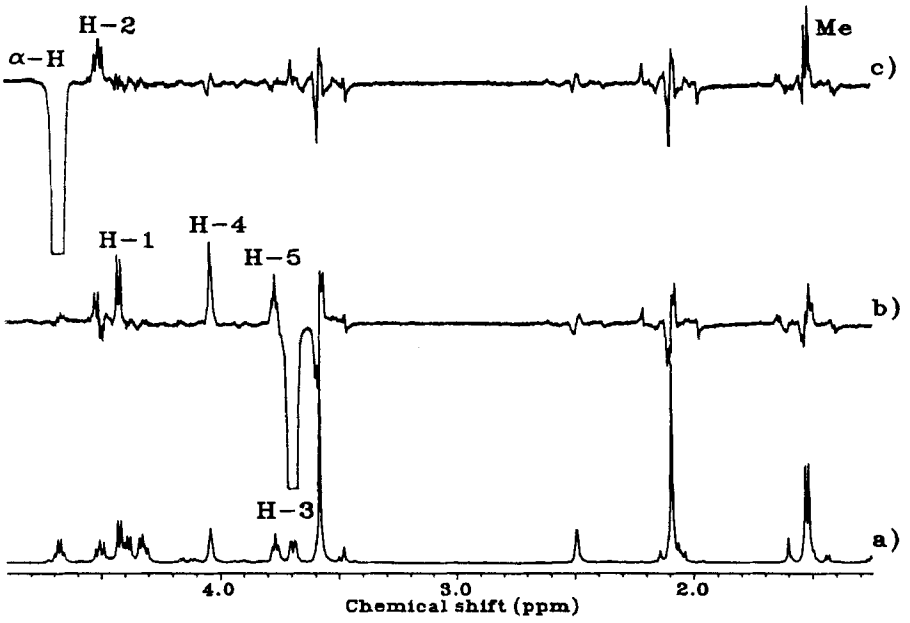



**Figure 1.** a)$^1$H NMR spectrum of compound **8a**, b)NOE difference spectrum obtained by irradiation of the H-3 resonance, and c)NOE difference spectrum obtained by irradiation of the lactyl α-H resonance.

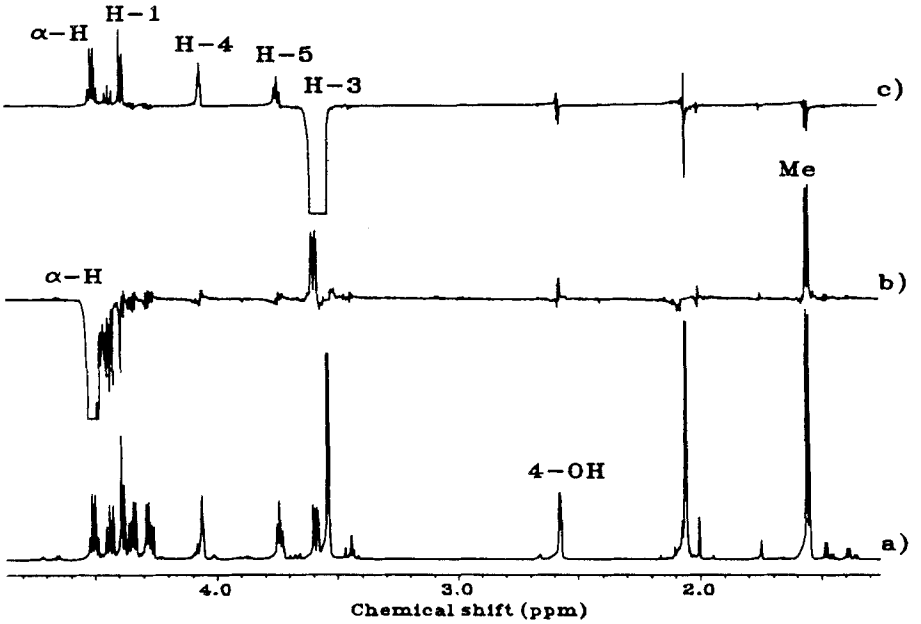
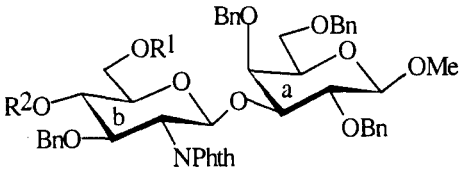


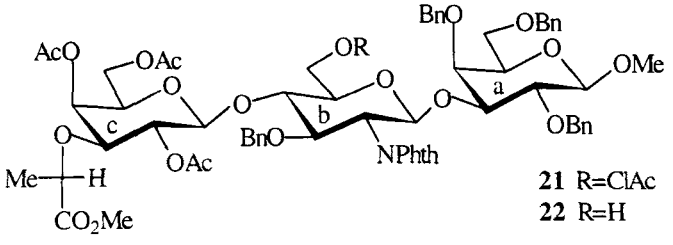

**Figure 2.** a)$^1$H NMR spectrum of compound **8b**, b)NOE difference spectrum obtained by irradiation of the lactyl α-H resonance, and c)NOE difference spectrum obtained by irradiation of the H-3 resonance.

tri-*O*-acetyl-3-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-D-galactopyranose (**13**). Compound **13** was then treated with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)[32] to produce galactosyl donor **14** in 41% yield from **12**. The overall yield of **14** from **11** was improved from 33% to 59% by hydrolysis of **11** directly with $CH_3CN/H_2O$ (10:1) in the presence of silver triflate and 2,6-lutidine to give **13** (90% yield), from which **14** was obtained by reaction with trichloroacetonitrile and DBU.

Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**15**)[33,34] has been previously synthesized using trimethyl(phenylthio)silane and trimethylsilyl triflate. We found that **15** could be prepared under the same conditions in >90% yield using inexpensive thiophenol by its reaction with 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside.[35] A major advantage of this method is that column chromatography was avoided; after removal of solvent, **15** was obtained by crystallization from methanol. Compound **16** was prepared from **15** in three steps,[33,34] and phenyl 3-*O*-benzyl-4,6-di-*O*-chloroacetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**17**) was then obtained from **16** by reaction with chloroacetic anhydride in 88% yield.




**18** $R^1=R^2=ClAc$
**19** $R^1=R^2=H$
**20** $R^1=ClAc$ $R^2=H$



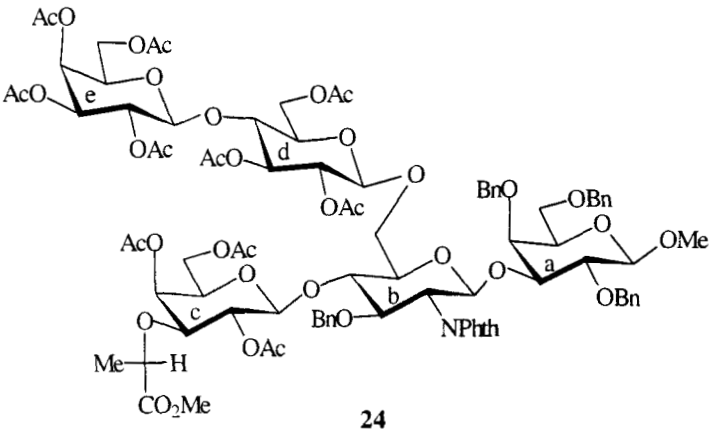

**21** R=ClAc
**22** R=H

Coupling of **17** with **4** was achieved using *N*-iodosuccinimide (NIS) and a catalytic amount of triflic acid (TfOH)[36,37] as activators, affording disaccharide derivative **18** in >90% yield. Removal of the chloroacetyl groups of compound **18**, using thiourea and 2,6-lutidine in methanol/dichloromethane,[38-40] gave 4,6-diol **19** in quantitative yield. Compound **19** was

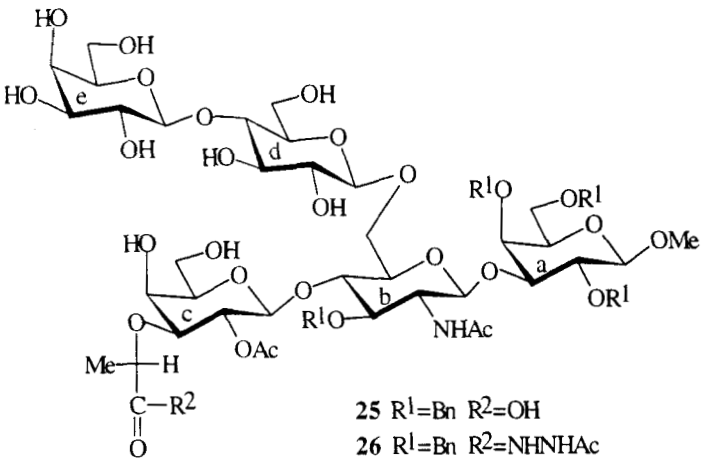
selectively chloroacetylated at the $6^b$-*O*-position by reaction with one equivalent of chloroacetic anhydride in the presence of 2,6-lutidine to furnish **20** in 82% yield. Disaccharide **20** was then condensed with compound **14,** using trimethysilyl triflate as an activator, to yield a trisaccharide **21**. Compound **21** was dechloroacetylated to furnish methyl *O*-{2,4,6-tri-*O*-acetyl-3-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-β-D-galactopyranosyl}-(1→4)-*O*-(3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**22**) in 92% yield. Subsequent condensation of **22** with lactosyl trichloroacetimidate **23**[20] gave pentasaccharide **24** in 69% yield.

Deprotection of **24** was performed by standard procedures: treatment of **24** with NaOMe/MeOH/$H_2O$ removed *O*-acetyl groups and caused deesterification.[41] The phthalimido group was removed by treatment with hydrazine hydrate, and the free amine obtained was *N*-acetylated with acetic anhydride in methanol[41] to afford a partially deprotected pentasaccharide, methyl *O*-(β-D-galactopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyl)-(1→6)-*O*-{3-*O*-[(*S*)-1-carboxyethyl]-*O*-β-D-galactopyranosyl-(1→4)-*O*}-(2-acetamido-3-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**25**). Structurally significant signals observed in the $^1$H NMR spectrum of pure **25** were a singlet at 1.66 ppm (NAc) and a doublet at 1.44 ppm (lactyl methyl). However, the presence of an additional singlet at 1.99 ppm and a doublet at 1.45 ppm indicated the presence of the hydrazide by-product **26**, which was formed by the reaction of carboxylic acid with hydrazine hydrate following *N*-acetylation. The ratio of **25** to **26** was about 5:1 according to the relative intensities of the above signals in the $^1$H NMR spectrum. The mixture of **25** and **26** was then hydrogenated to give a mixture of **27** and **28**.

Compounds **27** and **28** were separated by ion-exchange chromatography on Sephadex DEAE, using 10 mM Tris buffer (pH 7.5) to elute **28**, and then 10 mM Tris buffer with 0.5 M NaCl to recover **27**. Desalting was performed using a Sephadex G-10 column. The $^1$H NMR spectra of **27** and **28** differ mainly in the chemical shifts of H-$4^c$, α-H and $CH_3$ of the lactyl group. However, their $^{13}$C NMR spectra are very similar, the anomeric carbon signals being almost identical: 103.18, 103.32, 103.48, 103.59 and 104.57 in **27**; and 103.05, 103.21, 103.46, 103.60 and 104.57 in **28**. Data of the FABMS analysis in both negative and positive modes were also in accord with structures **27** and **28**.



**24**



**25** $R^1$=Bn $R^2$=OH
**26** $R^1$=Bn $R^2$=NHNHAc
**27** $R^1$=H $R^2$=$O^-Na^+$
**28** $R^1$=H $R^2$=NHNHAc



## EXPERIMENTAL

**General methods.** Melting points are uncorrected. Optical rotations were measured at room temperature with a Perkin-Elmer 243 polarimeter, using a 10-cm 1-mL cell. $^1H$ and $^{13}C$ NMR spectra were recorded at 500 MHz and 125 MHz, respectively, with a Bruker AMX 500 instrument at 300 K unless otherwise noted. Chemical shifts are given relative to the signal for internal $Me_4Si$ or indirectly to solvent signal 7.25 ($CDCl_3$),

4.76 ($CD_3OD$), 4.76 ($D_2O$) for $^1H$ NMR spectra, and to the solvent signals 76.9 ($CDCl_3$), 31.55 (internal acetone) for $^{13}C$ NMR spectra. The $^1H$ NMR resonances of oligosaccharides were assigned on the basis of 2D $^1H$-homonuclear chemical-shift correlated ($^1H$-COSY) experiments. FAB mass spectroscopic analyses were performed with a *JEOL JMS-AX505H* mass spectrometer.

Column chromatography was performed on Silica gel 60 (Merck, 230-400 mesh) and fractions were monitored by TLC on Silica gel 60 $F_{254}$ (Merck). Detection was effected by examination under UV light and by charring with 5% sulphuric acid solution in ethanol. Solutions were concentrated at or below 40 °C and dried with anhydrous $Na_2SO_4$.

**Methyl 2,4,6-Tri-*O*-benzyl-β-D-galactopyranoside (4).** To a solution of **3** (10.0 g, 20.0 mmol) in methanol (100 mL) was added $PdCl_2$ (4.0 g). The mixture was stirred at rt overnight until the starting material was consumed (TLC). The mixture was filtered through Celite, and the filtrate was concentrated and purified by chromatography (EtOAc/hexane 1:4) to afford crystalline **4** (8.1 g, 88%): mp 59-60 °C; lit.[21] mp 59-60 °C.

**Methyl 2,4,6-Tri-*O*-benzyl-3-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-β-D-galacto-pyranoside (5a) and Methyl 2,4,6-Tri-*O*-benzyl-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-β-D-galactopyranoside (5b).** Compound **4** (5.0 g, 10.76 mmol) was dissolved in dry DMF (50 mL), and after the addition of sodium hydride (1.0 g, 50% suspension in mineral oil) the mixture was stirred at 60 °C for 1 h. Methyl (*S*)-2-chloropropionate (5.0 g, 40.8 mmol) was added dropwise and stirring was continued at 60 °C for another 24 h. Upon cooling, the reaction was quenched by the addition of methanol, and the mixture was neutralized with 1N HCl. Water (100 mL) was added and the solution was extracted with ethyl acetate (200 mL). The ethyl acetate solution was washed with water (50 mL x 2), dried and concentrated to a syrup, which was fractionated by chromatography to afford a syrup **5a** (3.4 g, 57.2%) and its (*R*)-diastereomer **5b** (0.5 g, 8.4%).

For **5a**: $[\alpha]_D$ -25.7° (*c* 1.35, MeOH); $^1H$ NMR ($CDCl_3$) δ 1.367 (d, 3H, *Me*$CHCO_2Me$, J = 6.8 Hz), 3.395 (dd, 1H, H-3, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 2.8 Hz), 3.460 (dd, 1H, H-6a, $J_{6a,6b}$ = 9.1 Hz, $J_{5,6a}$ = 5.8 Hz), 3.518 (t, 1H, H-5, $J_{5,6}$ = 5.8 Hz), 3.530 (s, 3H, OMe), 3.585 (dd, 1H, H-6b, $J_{6a,6b}$ = 9.1 Hz, $J_{5,6b}$ = 5.8 Hz), 3.700 (s, 3H, $CO_2Me$), 3.762 (dd, 1H, H-2, $J_{2,3}$ = 9.5 Hz), 4.060 (d, 1H, H-4, $J_{3,4}$ = 2.8 Hz), 4.203 (d, 1H, H-1, $J_{1,2}$ = 7.8

Hz), 4.366 and 4.429 (2d, 1H each, C*H*$_2$Ph, J = 11.9 Hz), 4.453 (q, 1H, MeC*H*CO$_2$Me, J = 6.8 Hz), 4.652 and 4.681 (2d, 1H each, C*H*$_2$Ph, J = 11.8 Hz), 4.926 and 5.019 (2d, 1H each, C*H*$_2$Ph, J = 11.7 Hz), 7.271-7.378 (m, 15H, 3 x Ph).

For **5b**: $[\alpha]_D$ -7.0° (*c* 0.43, MeOH); $^1$H NMR ($CDCl_3$) δ 1.390 (d, 3H, *Me*CHCO$_2$Me, J = 6.7 Hz), 3.471 (s, 3H, OMe), 3.585 (s, 3H, CO$_2$Me), 3.842 (bs, 1H, H-4), 4.230 (d, 1H, H-1, $J_{1,2}$ = 7.5 Hz), 4.306 (q, 1H, MeC*H*CO$_2$Me, J = 6.7 Hz), 4.415 and 4.457 (2d, 1H each, C*H*$_2$Ph, J = 11.6 Hz), 4.599 and 4.742 (2d, 1H each, C*H*$_2$Ph, J = 11.8 Hz), 4.840 and 4.913 (2d, C*H*$_2$Ph, J = 11.7 Hz), 7.271-7.378 (m, 15H, 3 x Ph).

**Methyl 3-*O*-[(*S*)-1-(Methoxycarbonyl)ethyl]-β-D-galactopyranoside (6a).** To a solution of **5a** (3.3 g, 5.98 mmol) in methanol (30 mL) was added 10% palladium-on-carbon (50% water, 0.5 g). The mixture was stirred under hydrogen pressure (35-40 psi) for 3 h. The filtrate was concentrated to give crystals of **6a** (1.64 g, 97%): mp 141-3 °C (EtOAc/hexane); $[\alpha]_D$ -37.3° (*c* 0.55, MeOH); $^1$H NMR ($CDCl_3$) δ 1.470 (d, 3H, *Me*CHCO$_2$Me, J = 6.8 Hz), 2.226 (dd, 1H, 6-OH), 2.358 (bs, 1H, 2-OH), 3.329 (dd, 1H, H-3, $J_{2,3}$ = 9.2 Hz, $J_{3,4}$ = 3.2 Hz), 3.433 (bs, 1H, 4-OH), 3.506 (t, 1H, H-5, $J_{5,6}$ = 5.3 Hz), 3.550 (s, 3H, OMe), 3.751 (s, 3H, CO$_2$Me), 3.781 (dd 1H, H-2, $J_{2,3}$ = 9.2 Hz), 3.841 (m, 1H, H-6a), 3.945 (bs, 1H, H-4), 3.990 (m, 1H, H-6b), 4.152 (d, 1H, H-1, $J_{1,2}$ = 7.7 Hz), 4.248 (q, 1H, MeC*H*CO$_2$Me, J = 6.8 Hz); HRFABMS Calcd for $C_{11}H_{20}O_8$: 280.1158. Found: 281.1245 $[M+H]^+$.

Anal. Calcd for $C_{11}H_{20}O_8$: C, 47.1; H, 7.2. Found: C, 47.2; H, 7.2.

**Methyl 3-*O*-[(*R*)-1-(Methoxycarbonyl)ethyl]-β-D-galactopyranoside (6b).** Compound **6b** was prepared from **5b** as described for **6a**. Recrystallization from EtOAc/hexane gave crystals: mp 144-5 °C; $[\alpha]_D$ +26.9° (*c* 1.2, MeOH); $^1$H NMR ($CDCl_3$) δ 1.468 (d, 3H, *Me*CHCO$_2$Me, J = 6.9 Hz), 3.302 (dd, 1H, H-3, $J_{2,3}$ = 9.4 Hz, $J_{3,4}$ = 2.7 Hz), 3.472 (t, 1H, H-5, $J_{5,6}$ = 5.8 Hz), 3.561 (s, 3H, OMe), 3.760 (s, 3H, CO$_2$Me), 3.850 (m, 1H, H-6a), 3.951 (dd, 1H, H-6b, $J_{6,6}$ = 11.7 Hz, $J_{5,6}$ = 5.8 Hz), 3.989 (d, 1H, H-4, $J_{3,4}$ = 2.7 Hz), 4.160 (d, 1H, H-1, $J_{1,2}$ = 7.7 Hz), 4.222 (q, 1H, MeC*H*CO$_2$Me, J = 6.9 Hz); HRFABMS Calcd for $C_{11}H_{20}O_8$: 280.1158. Found: 281.1234 $[M+H]^+$.

Anal. Calcd for $C_{11}H_{20}O_8$: C, 47.1; H, 7.2. Found: C, 47.5; H, 7.3.

**Methyl 3-*O*-[(*S*)-1-Carboxyethyl]-β-D-galactopyranoside (7a) and lactone derivative of methyl 6-*O*-acetyl-3-*O*-[(*S*)-1-carboxyethyl]-β-D-galactopyranoside (8a).**

A solution of **6a** (30 mg) in 0.1 N NaOH (1 mL) was kept at 80 °C for 5 h. Upon cooling, the solution was neutralized by the addition of Dowex-50 ($H^+$) ion-exchange resin. The filtrate was lyophilized to give **7a** in quantitative yield: $^1$H NMR ($D_2O$) δ 1.416 (d, 3H, CH*Me*$CO_2$H, J = 6.8 Hz), 3.523 (dd, 1H, H-3, $J_{2,3}$ = 9.5 Hz), 3.575 (s, 3H, OMe), 3.613 (dd, 1H, H-2, $J_{2,3}$ = 9.5 Hz), 3.691 (q, 1H, H-5, $J_{5,6a}$ = 4.2 Hz, $J_{5,6b}$ = 7.2 Hz), 3.740-3.820 (m, 2H, H-6a, 6b), 4.023 (bs, 1H, H-4), 4.179 (q, 1H, C*H*MeCO$_2$H, J = 6.8 Hz), 4.349 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz).

Acetic anhydride (0.5 mL) was added to a suspension of **7a** in acetonitrile (1 mL), and the mixture was stirred at rt for 12 h until only one main product appeared on TLC. The mixture was concentrated by co-evaporation with toluene. Further purification by chromatography (EtOAc) gave compound **8a**: $^1$H NMR ($CDCl_3$) δ 1.507 (d, 3H, CH*Me*$CO_2$, J = 6.9 Hz), 2.071 (s, 3H, 6-OAc), 2.466 (bs, 1H, 4-OH), 3.559 (s, 3H, OMe), 3.678 (dd, 1H, H-3, $J_{2,3}$ = 9.5 Hz), 3.745 (t, 1H, H-5, $J_{5,6}$ = 6.5 Hz), 4.017 (bs, 1H, H-4), 4.317 and 4.368 (2q, 1H each, H-6a, 6b, $J_{6a,6b}$ = 11.3 Hz, $J_{5,6}$ = 6.5 Hz), 4.407 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 4.484 (dd, 1H, H-2, $J_{2,3}$ = 9.5 Hz), 4.662 (q, 1H, C*H*MeCO$_2$, J = 6.9 Hz); $^{13}$C NMR ($CDCl_3$) δ 17.63 (CH*Me*), 20.77 (6-OAc), 57.10 (OMe), 62.30, 66.88, 70.95, 72.32, 72.68, 75.12, 100.99 (C-1), 169.10, 170.80 (6-OAc and CHMe*C*OO).

**Methyl 3-*O*-[(*R*)-1-Carboxyethyl]-β-D-galactopyranoside (7b) and lactone derivative of methyl 6-*O*-acetyl-3-*O*-[(*R*)-1-carboxyethyl]-β-D-galactopyranoside (8b).** A solution of **6b** (100 mg) in 0.1 N NaOH (2 mL) was kept at 80 °C for 5 h, cooled and neutralized by the addition of Dowex-50 ($H^+$) ion-exchange resin. The filtrate was lyophilized to give **7b** in quantitative yield: $^1$H NMR ($D_2O$) δ 1.436 (d, 3H, CH*Me*$CO_2$H, J = 6.5 Hz), 3.544-3.617 (m, 2H, H-2, 3), 3.577 (s, 3H, OMe), 3.658 (m, 1H, H-5), 3.767 (m, 2H, H-6a, 6b), 4.110 (bs, 1H, H-4), 4.255 (q, 1H, C*H*MeCO$_2$H, J = 6.5 Hz), 4.324 (d, 1H, H-1, $J_{1,2}$ = 6.6 Hz).

To a suspension of **7b** in acetonitrile (2 mL) was added acetic anhydride (0.5 mL). The mixture was stirred at room temperature for 12 h until only one spot appeared on TLC. After concentration by co-evaporation with toluene, purification by chromatography (EtOAc) gave compound **8b**: $^1$H NMR ($CDCl_3$) δ 1.552 (d, 3H, CH*Me*$CO_2$, J = 6.9 Hz), 2.057 (s, 3H, 6-OAc), 2.504 (d, 1H, 4-OH, J = 2.4 Hz), 3.540 (s, 3H, OMe), 3.594 (dd, 1H, H-3, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 2.7 Hz), 3.740 (t, 1H, H-5, $J_{5,6}$ = 6.5 Hz), 4.059 (bs, 1H, H-

4), 4.286 and 4.335 (2q, 1H each, H-6a, 6b, $J_{6a,6b}$ = 11.3 Hz, $J_{5,6}$ = 6.5 Hz), 4.376 (d, 1H, H-1, $J_{1,2}$ = 8.1 Hz), 4.439 (dd, 1H, H-2, $J_{2,3}$ = 9.5 Hz), 4.514 (q, 1H, C*H*Me, J = 6.9 Hz); $^{13}$C NMR ($CDCl_3$) δ 18.50 (CH*Me*), 20.75 (6-OAc), 56.99 (OMe), 62.34, 66.66, 72.44, 73.74, 74.60, 75.81, 100.93 (C-1), 168.60, 170.87 (6-OAc and CHMe*C*OO).

**Methyl 2,4,6-Tri-*O*-acetyl-3-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-β-D-galacto-pyranoside (9).** Acetic anhydride (5 mL) was added to a solution of compound **6a** (1.6 g, 5.67 mmol) in dry pyridine (10 mL). The mixture was stirred overnight, poured into ice-water, and extracted with dichloromethane (20 mL x 2). The organic phase was washed with water, dried and concentrated, to give **9** (2.3 g, 99%): mp 98-99 °C (EtOAc/hexane); $[\alpha]_D$ +8.9° (*c* 0.79, MeOH); $^1$H NMR ($CDCl_3$) δ 1.311 (d, 3H, *Me*CHCO$_2$Me, J = 6.7 Hz), 2.045, 2.075, 2.111 (3s, 3H each, 3 x OAc), 3.470 (s, 3H, OMe), 3.594 (dd, 1H, H-3, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 3.1 Hz), 3.700 (s, 3H, $CO_2$Me), 3.786 (t, 1H, H-5, $J_{5,6}$ = 6.2 Hz), 4.069-4.166 (m, 3H, H-6a, 6b, MeC*H*CO$_2$Me), 4.293 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 5.117 (dd, 1H, H-2, $J_{2,3}$ = 9.5 Hz), 5.508 (d, 1H, H-4); HRFABMS Calcd for $C_{17}H_{26}O_{11}$: 406.1475. Found: 407.1539 $[M+H]^+$, 406.1476 $[M]^+$.

Anal. Calcd for $C_{17}H_{26}O_{11}$: C, 50.2; H, 6.5. Found: C, 49.7; H, 6.4.

**Methyl 2,6-Di-*O*-acetyl-3-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-β-D-galacto-pyranoside (10).** Compound **6a** was treated with acetic anhydride at room temperature for 2 h, giving **10**: $^1$H NMR ($CDCl_3$) δ 1.388 (d, 3H, *Me*CHCO$_2$Me, J = 6.8 Hz), 2.063, 2.072 (2s, 3H each, 2 x OAc), 3.335 (bs, 1H, 4-OH), 3.406 (dd, 1H, H-3, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 2.8 Hz), 3.456 (s, 3H, OMe), 3.641 (t, 1H, H-5, $J_{5,6}$ = 5.8 Hz), 3.743 (s, 3H, $CO_2$Me), 3.940 (bs, 1H, H-4), 4.031 (q, 1H, MeC*H*CO$_2$Me, J = 6.8 Hz), 4.267 (d, 1H, H-1, $J_{1,2}$ = 8.1 Hz), 4.348-4.369 (m, 2H, H-6a, 6b), 5.171 (dd, 1H, H-2, $J_{2,3}$ = 9.6 Hz).

**2,4,6-Tri-*O*-acetyl-3-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-α-D-galactopyranosyl chloride (11).** To a solution of **9** (2.3 g, 5.66 mmol) and 1,1-dichloromethyl methyl ether (10 mL) in dichloromethane (30 mL) was added zinc chloride (20 mg). The mixture was stirred at rt for 2 h until TLC indicated that the starting material was converted to a fast-moving product. The filtrate was washed successively with cold water, aq $NaHCO_3$, and water, dried and concentrated to a brown syrup. Purification by column chromatography (EtOAc/hexane 1:1) afforded a colourless syrup **11** (2.2 g, 94.6%): $^1$H NMR ($CDCl_3$) δ 1.351 (d, 3H, *Me*CHCO$_2$Me, J = 6.8 Hz), 2.038 (s, 3H, OAc), 2.095 (s, 6H, 2 x OAc),

3.717 (s, 3H, $CO_2Me$), 3.962 (dd, 1H, H-3, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 2.8 Hz), 4.028 (dd, 1H, H-6a, $J_{6a,6b}$ = 11.5 Hz, $J_{5,6}$ = 6.1 Hz), 4.109-4.195 (m, 2H, H-6b, Me*C*H$CO_2$Me), 4.385 (t, 1H, H-5, $J_{5,6}$ = 6.1 Hz), 5.114 (dd, 1H, H-2, $J_{2,3}$ = 10.1 Hz), 5.636 (d, 1H, H-4), 6.359 (d, 1H, H-1, $J_{1,2}$ = 3.75 Hz).

**1,2,4,6-Tetra-*O*-acetyl-3-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-β-D-galactopyranose (12).** To a solution of **11** (2.2 g, 5.36 mmol) in acetic acid (30 mL) were added silver triflate (2.4 g, 9.34 mmol) and 2,6-lutidine (1 mL). The mixture was stirred at rt for 2 h until TLC indicated that the reaction was complete. The filtrate was concentrated by repeated coevaporation with toluene to afford a syrup, which was purified by column chromatography (EtOAc/hexane 1:1) to give **12** (2.0 g, 85.2%): $^1$H NMR ($CDCl_3$) δ 1.275 (d, 3H, *Me*CHCO$_2$Me, J = 6.6 Hz), 1.984, 2.004, 2.038, 2.074 (4s, 3H each, 4 x OAc), 3.658 (s, 3H, $CO_2Me$), 3.677 (m, 1H, H-3), 3.900 (t, 1H, H-5, $J_{5,6}$ = 5.9 Hz), 4.001 (dd, 1H, H-6a, $J_{6a,6b}$ = 10.7 Hz, $J_{5,6}$ = 5.9 Hz), 4.043 (q, 1H, Me*C*H$CO_2$Me, J = 6.6 Hz), 4.121 (dd, 1H, H-6b), 5.189 (dd, 1H, H-2, $J_{2,3}$ = 9.1 Hz), 5.493 (bs, 1H, H-4), 5.564 (d, 1H, H-1, $J_{1,2}$ = 8.2 Hz).

**2,4,6-Tri-*O*-acetyl-3-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-α-D-galactopyranosyl trichloroacetimidate (14). Method 1**: To a solution of **12** (2.0 g, 4.56 mmol) in DMF (10 mL) was added hydrazine acetate (0.6 g, 6.52 mmol). The mixture was stirred at 60 °C for 2 h and, after cooling to rt, extracted with ethyl acetate (100 mL). The extract was washed with water, 0.5 N HCl, and water, dried and concentrated to a syrup **13** (1.6 g). To a solution of the above syrup in dichloromethane (30 mL) and trichloroacetonitrile (4 mL, 10 mmol) at 0 °C was added DBU (0.7 mL, 4.67 mmol). The mixture was stirred at 0 °C until TLC indicated that the reaction was complete (ca. 2 h). The mixture was concentrated and the remaining syrup was fractionated by column chromatography (EtOAc/hexane 1:1) to give a solid residue (**14**, 1.0 g, 40.9%). Recrystallization from EtOAc/hexane gave needles of **14** (0.8 g): mp 92-93 °C; $[\alpha]_D$ +109° (*c* 0.79, MeOH); $^1$H NMR ($CDCl_3$) δ 1.356 (d, 3H, CH*Me*$CO_2$, J = 6.7 Hz), 2.004, 2.017, 2.127 (3s, 3H each, 3 x OAc), 3.725 (s, 3H, $CO_2Me$), 4.002 (m, 2H, H-3,6a), 4.176 (m, 2H, H-6b, *CH*MeCO$_2$), 4.310 (t, 1H, H-5, $J_{5,6}$ = 5.8 Hz), 5.240 (dd, 1H, H-2, $J_{2,3}$ = 10.2 Hz), 5.648 (d, 1H, H-4, $J_{3,4}$ = 2.4 Hz), 6.579 (d, 1H, H-1, $J_{1,2}$ = 3.3 Hz), 8.615 (s, 1H, NH); HRFABMS Calcd for $C_{18}Cl_3H_{24}NO_{11}Li$ (M+Li): 542.0575. Found: 542.0574.

Anal. Calcd for $C_{18}Cl_3H_{24}NO_{11}$: C, 40.3; H, 4.5; N, 2.6. Found: C, 40.0; H, 4.6; N, 2.7.

**Method 2**: To a solution of **11** (0.91 g, 2.22 mmol) in acetonitrile/water (10:1, 10 mL) was added silver triflate (0.7 g, 2.72 mmol) and 2,6-lutidine (0.5 mL). The mixture was stirred at rt until TLC indicated complete transformation of the starting material (overnight). The reaction mixture was diluted with dichloromethane (50 mL), washed successively with water, aq $NaHCO_3$, and water, dried and concentrated to give **13** (0.78 g, 1.99 mmol, 90%). This syrup was dissoled in dichloromethane (30 mL), treated with trichloroacetonitrile (2 mL) and DBU (0.3 mL, 2.0 mmole), as described in method 1, to give needles of **14** (0.7 g, 58.9%).

**Phenyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (15).** To a solution of 1,3,4,6-tetra-*O*-acetyl-2-phthalimido-2-deoxy-β-D-glucopyranose[35] (10 g, 26.7 mmol) in dichloromethane (100 mL) was added thiophenol (5 mL) and trimethylsilyl triflate (5 mL). The mixture was stirred at rt for 10 h. After neutralization with triethylamine, the solution was washed successively with water, aq $NaHCO_3$, and water, dried and concentrated. The residue was crystallized from methanol (50 mL), to give crystals of **15** (9.4 g, 85.1%). Another 1.2 g of the same product was recovered from the mother liquor (total yield 98%): mp 144-145 °C; $[\alpha]_D$ +60.9° (*c* 1.45, $CH_2Cl_2$); lit.[33,34] mp 145-146 °C; $[\alpha]_D$ +53.0°,[33] +56.1°;[34] $^1$H NMR ($CDCl_3$) δ 1.815, 2.001, 2.080 (3s, 3H each, 3 x OAc), 3.873 (m, 1H, H-5), 4.176 (d, 1H, H-6a, $J_{6a,6b}$ = 12.1 Hz), 4.273 (dd, 1H, H-6b, $J_{5,6b}$ = 5.0 Hz, $J_{6a,6b}$ = 12.1 Hz), 4.332 (t, 1H, H-2, $J_{1,2} = J_{2,3}$ = 9.8 Hz), 5.116 (t, 1H, H-4, $J_{3,4} = J_{4,5}$ = 9.8 Hz), 5.683 (d, 1H, H-1, $J_{1,2}$ = 9.8 Hz), 5.774 (t, 1H, H-3, $J_{2,3} = J_{3,4}$ = 9.8 Hz), 7.239-7.398 (m, 5H, SPh), 7.735-7.847 (m, 4H, Phth).

**Phenyl 3-*O*-Benzyl-4,6-di-*O*-chloroacetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (17).** To a solution of **16**[33,34] (1.9 g, 3.87 mmol) in dichloromethane (50 mL) and 2,6-lutidine (2 mL) was added chloroacetic anhydride (2.0 g, 11.70 mmol). The mixture was stirred at rt overnight and then washed successively with ice-cold water, 1N HCl, water, aq $NaHCO_3$, and water. Concentration gave amorphous **17** (2.2 g, 88.3%): $^1$H NMR ($CDCl_3$) δ 3.852 (m, 1H, H-5), 3.869 and 3.934 (2d, 1H each, 4-ClAc, J = 14.6 Hz), 4.071 (s, 2H, 6-ClAc), 4.271-4.355 (m, 4H, H-3, 6a, 6b, and one of $CH_2$Ph), 4.482 (t, 1H, H-2, $J_{1,2} = J_{2,3}$ = 9.3 Hz), 4.545 (d, 1H, one of $CH_2$Ph, J = 12.1 Hz), 5.155 (t, 1H,

H-4, $J_{3,4} = J_{4,5} = 9.6$ Hz), 5.509 (d, 1H, H-1, $J_{1,2} = 9.3$ Hz), 6.905-6.978 (m, 5H, Ph), 7.239-7.351 (m, 5H, SPh), 7.704 (m, 4H, Phth).

**Methyl *O*-(3-*O*-Benzyl-4,6-di-*O*-chloroacetyl-2-deoxy-2-phthalimido-β-D-gluco-pyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (18).** To a solution of **4** (0.9 g, 1.93 mmol), **17** (1.0 g, 1.55 mmol) and NIS (0.9 g) in dichloromethane (10 mL) were added 4Å molecular sieves (1.0 g). The mixture was stirred at rt for 1 h. The solution was then cooled to -45 °C and triflic acid (ca. 25μL) was added under nitrogen. The mixture was stirred at -45 °C for another 1 h and the reaction was then quenched by the addition of a solution of 2,6-lutidine (1 mL) in dichloromethane (20 mL). The filtrate was washed successively with cold water, 1N HCl, water, dried and concentrated to a residue. Purification by column chromatography (EtOAc/hexane 1:1) gave amorphous **18** (1.5 g, 97%): $[\alpha]_D$ +20° (*c* 1.45, MeOH); $^1$H NMR ($CDCl_3$) δ 3.331 (s, 3H, OMe), 3.851 and 3.895 (2d, 1H each, $4^b$-ClAc, J = 14.5 Hz), 3.967 (s, 2H, $6^b$-ClAc), 4.112 (d, 1H, H-$1^a$, $J_{1,2} = 7.7$ Hz), 5.135 (t, 1H, H-$4^b$, $J_{3,4} = J_{4,5} = 9.3$ Hz), 5.481 (d, 1H, H-$1^b$, $J_{1,2} = 8.6$ Hz), 6.910-7.238 (m, 20H, 4 x Ph), 7.520 (m, 4H, Phth); HRFABMS Calcd for $C_{53}Cl_2H_{53}NO_{14}Li$ (M+Li): 1004.3003. Found: 1004.2988.

**Methyl *O*-(3-*O*-Benzyl-6-*O*-chloroacetyl-2-deoxy-2-phthalimido-β-D-gluco-pyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (20).** To a solution of compound **18** (1.2 g, 1.20 mmol) in dichloromethane/methanol (1:1, 30 mL) was added thiourea (0.5 g) and 2,6-lutidine (0.5 mL). The mixture was stirred at rt until TLC indicated that the reaction was complete (36 h). Ethyl acetate (100 mL) was added and the solution was successively washed with water, 1N HCl, and water. The organic layer was dried and concentrated to give amorphous **19** (1.01 g, 99.6%): $[\alpha]_D$ +13.4° (*c* 0.77, MeOH); $^1$H NMR ($CDCl_3$) δ 1.718 (bs, 1H, $6^b$-OH), 2.319 (bs, 1H, $4^b$-OH), 3.324 (s, 3H, OMe), 4.116 (d, 1H, H-$1^a$, $J_{1,2} = 7.2$ Hz), 5.501 (d, 1H, H-$1^b$, $J_{1,2} = 8.3$ Hz), 6.975-7.391 (m, 20H, 4 x Ph), 7.392-7.553 (m, 4H, Phth).

To a solution of **19** (1.01 g, 1.19 mmol) and 2,6-lutidine (1 mL) in dichloromethane (30 mL) at 0 °C was added dropwise a solution of chloroacetic anhydride (220 mg, 1.29 mmol) in dichloromethane (5 mL). The mixture was stirred at rt until TLC indicated the formation of one major product (**20**) (overnight). The solution was washed successively with cold water, 1N HCl, and water, dried and concentrated. The residue

was purified by column chromatography (EtOAc/hexane 1:1) to give amorphous **20** (0.9 g, 82.1%): $[\alpha]_D$ +15.4° (*c* 0.55, MeOH); $^1$H NMR ($CDCl_3$) δ 2.678 (bs, 1H, 4$^b$-OH), 3.339 (s, 3H, OMe), 3.955 (s, 2H, 6$^b$-ClAc), 4.119 (d, 1H, H-1$^a$, $J_{1,2}$ = 7.5 Hz), 5.487 (d, 1H, H-1$^b$, $J_{1,2}$ = 7.9 Hz), 6.964-7.667 (m, 24H, 4 x Ph and Phth); $^{13}$C NMR ($CDCl_3$) δ 40.60 ($ClCH_2CO$), 55.96 (C-2$^b$), 56.86 (OMe), 99.43 (C-1$^a$), 104.91 (C-1$^b$), 123.30, 131.22, 131.41, 133.77 (Phth), 126.94-138.95 (4 x Ph), 167.61-168.24 ($ClCH_2CO$ and Phth); HRFABMS Calcd for $C_{49}H_{51}NO_{12}Li$ (M+Li): 928.3287. Found: 928.3290.

**Methyl *O*-{2,4,6-Tri-*O*-acetyl-3-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-β-D-galacto-pyranosyl}-(1→4)-*O*-(3-*O*-benzyl-6-*O*-chloroacetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (21).** To a solution of **14** (130 mg, 0.24 mmol) and **20** (180 mg, 0.20 mmol) in dichloromethane (5 mL) were added 4Å molecular sieves (250 mg). The mixture was stirred at rt for 1 h. Trimethylsilyl triflate (50 μL) was added at -45 °C and the mixture was stirred at that temperature for 2 h. The reaction was quenched by the addition of a solution of 2,6-lutidine (0.5 mL) in dichloromethane (50 mL). The filtrate was washed successively with cold water, 1N HCl, and water, dried, and concentrated to a residue that was purified by chromatography (EtOAc/hexane 1:1) to give amorphous **21** (160 mg, 63%): $[\alpha]_D$ +26.2° (*c* 0.29, MeOH); $^1$H NMR ($CDCl_3$) δ 1.316 (d, 3H, $CHMeCO_2Me$, J = 6.8 Hz), 1.990, 2.046, 2.115 (3s, 3H each, 3 x OAc), 3.318 (s, 3H, OMe), 3.698 (s, 3H, $CO_2Me$), 3.917 (s, 2H, $ClCH_2CO$), 4.099 (d, 1H, H-1$^a$, $J_{1,2}$ = 7.6 Hz), 4.504 (d, 1H, H-1$^c$, $J_{1,2}$ = 8.1 Hz), 5.179 (dd, 1H, H-2$^c$, $J_{2,3}$ = 8.4 Hz), 5.457 (d, 1H, H-1$^b$, $J_{1,2}$ = 8.5 Hz), 5.472 (bs, 1H, H-4$^c$), 6.798-7.472 (m, 24H, 4 x Ph and Phth); $^{13}$C NMR ($CDCl_3$) δ 18.04 (*CHMe*), 20.68, 20.84, 21.30 (3 x $CH_3CO$), 40.48 ($ClCH_2CO$), 52.06 (C-2$^b$), 56.10, 56.82 (OMe and $CO_2Me$), 99.12 (C-1$^a$), 101.18 (C-1$^c$), 104.89 (C-1$^b$), 123.15, 131.33, 133.53 (Phth), 126.99-128.55 and 137.95-138.94 (4 x Ph).

**Methyl *O*-{2,4,6-Tri-*O*-acetyl-3-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-β-D-galacto-pyranosyl}-(1→4)-*O*-(3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (22).** To a solution of **21** (150 mg, 0.12 mmol) in dichloromethane/methanol (1:1, 3 mL) was added thiourea (130 mg, 1.71 mmol) and 2,6-lutidine (0.5 mL). The mixture was stirred at rt until the conversion was complete (ca. 24 h). Ethyl acetate (20 mL) was added and the solution was processed as

described for the preparation of **19** to give amorphous **22** (130 mg, 92%): $[\alpha]_D$ +11.8° (*c* 0.88, MeOH); $^1$H NMR ($CDCl_3$) δ 1.311 (d, 3H, CH*Me*$CO_2$Me, J = 6.8 Hz), 1.624 (bs, 1H, 6$^b$-OH), 1.981, 2.051, 2.090 (3s, 3H each, 3 x OAc), 3.327 (s, 3H, OMe), 3.696 (s, 3H, $CO_2$Me), 4.103 (d, 1H, H-1$^a$, $J_{1,2}$ = 7.2 Hz), 4.653 (d, 1H, H-1$^c$, $J_{1,2}$ = 8.1 Hz), 5.170 (dd, 1H, H-2$^c$, $J_{2,3}$ = 9.0 Hz), 5.470 (bs, 1H, H-4$^c$), 5.487 (d, 1H, H-1$^b$, $J_{1,2}$ = 8.7 Hz), 6.808-7.499 (m, 24H, 4 x Ph and Phth); $^{13}$C NMR ($CDCl_3$) δ 18.04 (CH*Me*), 20.67 (2 x *C*$H_3$CO), 20.88 (*C*$H_3$CO), 52.03 (C-2$^b$), 56.24, 56.83 (OMe, $CO_2$*Me*), 98.86 (C-1$^a$), 101.03 (C-1$^c$), 104.96 (C-1$^b$), 123.14-138.77 (4 x Ph), 167.67 (C=O of Phth), 168.95, 170.08, 170.42 (3 x $CH_3$*C*O), 172.28 (*C*$O_2$Me).

**Methyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-{2,4,6-tri-*O*-acetyl-3-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-β-D-galactopyranosyl-(1→4)-*O*}-(3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (24).** To a solution of **23** (120 mg, 0.15 mmol) and **22** (120 mg, 0.10 mmol) in dichloromethane (5 mL) were added 4Å molecular sieves (270 mg). The mixture was stirred at rt for 1 h, then cooled to -45 °C. Trimethyl triflate (40 μL) was added and stirring was continued at the same temperature for another 2 h. The reaction was quenched by the addition of a solution of 2,6-lutidine (0.5 mL) in dichloromethane (40 mL), and the mixture was processed as described for the preparation of **21**. Purification by chromatography (EtOAc/Hexane 2:1) gave product **24** (125 mg, 69%) as an amorphous solid: $[\alpha]_D$ +4.2° (*c* 0.72, MeOH); $^1$H NMR ($CDCl_3$) δ 1.324 (d, 3H, CH*Me*$CO_2$Me, J = 6.6 Hz), 1.910, 1.929, 1.964, 1.975, 2.015, 2.031, 2.055, 2.095, 2.099, 2.120 (10s, 3H each, 10 x OAc), 3.328 (s, 3H, OMe), 3.704 (s, 3H, $CO_2$Me), 4.652 (d, 1H, H-1$^d$, $J_{1,2}$ = 7.6 Hz), 4.925 (dd, 1H, H-3$^e$, $J_{2,3}$ = 9.3 Hz), 5.072 (dd, 1H, H-2$^e$), 5.162 (dd, 1H, H-2$^c$, $J_{2,3}$ = 9.0 Hz), 5.237 (dd, 1H, H-3$^d$, $J_{2,3}$ = 8.4 Hz, $J_{3,4}$ = 9.1 Hz), 5.311 (bs, 1H, H-4$^e$), 5.381 (d, 1H, H-1$^b$, $J_{1,2}$ = 8.3 Hz), 5.471 (bs, 1H, H-4$^c$), 6.801-7.537 (m, 24H, 4 x Ph and Phth); $^{13}$C NMR ($CDCl_3$) δ 18.097 (CH*Me*), 20.46-20.92 (10 x $CH_3$CO), 52.06 (C-2$^b$), 56.21, 56.81 (OMe, $CO_2$*Me*), 98.84 (C-1$^a$), 101.02 (C-1$^c$), 101.22(C-1$^d$ and C-1$^e$), 105.00 (C-1$^b$), 123.14-138.94 (4 x Ph), 168.97-172.31 (10 x $CH_3$*C*O, *C*$O_2$Me, and Phth).

**Methyl *O*-(β-D-Galactopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyl)-(1→6)-*O*-{3-*O*-[(*S*)-1-carboxyethyl]-β-D-galactopyranosyl-(1→4)-*O*}-(2-acetamido-3-*O*-benzyl-2-**

**deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (25) and Methyl *O*-(β-D-Galactopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyl)-(1→6)-*O*-{3-*O*-[(*S*)-1-(*N*-acetylhydrazidocarbonyl)ethyl]-β-D-galactopyranosyl-(1→4)-*O*}-(2-acetamido-3-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (26).** To a solution of **24** (100 mg, 0.054 mmol) in methanol (5 mL) was added 0.5% NaOMe/MeOH (1 mL) and water (0.1 mL). The mixture was stirred for 20 h and neutralized with Dowex-50 ($H^+$). The filtrate was concentrated to a residue and hydrazine hydrate (1 mL) was added to a solution of the residue in 95% ethanol (7 mL). The reaction mixture was refluxed for 20 h. After evaporation of the solvent, the residue was dissolved in methanol (5 mL) and stirred with acetic anhydride (1 mL) for 24 h. The solvent was then co-evaporated with toluene. The product was purified on a column of Sephadex LH-20, with methanol as eluent, to afford a mixture of **25** and **26** as an amorphous solid (50 mg, 70%). The ratio of **25** to **26** was about 5:1.

For **25**: $^1$H NMR ($CD_3OD$) δ 1.435 (d, 3H, CH*Me*$CO_2$Me, J = 7.0 Hz), 1.663 (s, 3H, NH*Ac*), 3.374 (s, 3H, OMe), 4.270 (d, 1H, H-1, $J_{1,2}$ = 7.6 Hz), 4.322 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 4.374 (d, 1H, H-1, $J_{1,2}$ = 7.7 Hz), 5.048 (d, 1H, H-1, $J_{1,2}$ = 8.1 Hz), 7.223-7.370 (m, 20H, 4 x Ph).

For **26**: 1.450 (d, 3H, CH*Me*CONHNH), 1.664 (s, 3H, N*Ac*), 1.994 (s, 3H, NHNH*Ac*).

**Methyl *O*-(β-D-Galactopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyl)-(1→6)-*O*-{3-*O*-[(*S*)-1-carboxyethyl]-β-D-galactopyranosyl-(1→4)-*O*}-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside (27) and Methyl *O*-(β-D-Galactopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyl)-(1→6)-*O*-{3-*O*-[(*S*)-1-(*N*-acetylhydrazidocarbonyl)ethyl]-β-D-galactopyranosyl-(1→4)-*O*}-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside (28).** A mixture of **25** and **26** (40 mg, 0.03 mmol), and 10% Pd-on-carbon (50% wet, 60 mg) in methanol/water/acetic acid (1:1:1, 8 mL) was hydrogenated (40 psi) for 12 h. The filtrate was lyophilized to give an amorphous solid (30 mg), which was dissolved in 0.1 N NaOH (3 mL) and kept for 5 h. The solution was then passed through a column of Sephadex G-10 to afford, after lyophilization, an amorphous solid (22 mg, 76.8%), that was shown by $^1$H NMR to contain mainly compound **27** together with **28** in a ratio of about 5:1. The separation of

**27** from **28** was achieved on a column of Sephadex DEAE. Compound **28** was eluted with 0.01 M tris buffer (pH 7.5) without NaCl, and compound **27** was then eluted with same buffer with 0.5 M NaCl. The two compounds were separately desalted by passage through a column of Sephadex G-10, and lyophilized to afford pure **27** (11 mg) and **28** (2 mg).

For **27**: $[\alpha]_D$ +26.4° (*c* 0.13, $H_2O$); $^1H$ NMR ($D_2O$, 293 K) δ 1.439 (d, 3H, CH*Me*$CO_2$, J = 6.4 Hz), 2.030 (s, 3H, NH*Ac*), 3.371 (dd, 1H, H-$2^d$), 3.565 (s, 3H, OMe), 3.898 (bs, 1H, H-$4^e$), 4.050 (bs, 1H, H-$4^c$), 4.145 (bs, 1H, H-$4^a$), 4.257 (q, 1H, C*H*MeCO$_2$, J = 6.4 Hz), 4.291 (d, 1H, H-$1^a$, $J_{1,2}$ = 8.6 Hz), 4.466 (d, 1H, H-$1^e$, $J_{1,2}$ = 7.5 Hz), 4.564 (d, 2H, H-$1^c$ and H-$1^d$, $J_{1,2}$ = 7.8 Hz), 4.729 (d, 1H, H-$1^b$, $J_{1,2}$= 8.1 Hz); $^{13}C$ NMR ($D_2O$) δ 18.87 (CH*Me*), 22.84 (*C*$H_3$CON), 55.88 (C-$2^b$), 57.87 (OMe), 103.18, 103.32, 103.48, 103.59, 104.57 (5 x C-1), 175.63 (CH$_3$*C*ON), 180.50 (COO$^-$Na$^+$); FABMS for $C_{36}H_{60}NO_{28}Na$ (977.8): *m/z* 977.5 $[M]^+$, 955.7 $[M+H-Na]^+$ (positive mode); 954.3 $[M-Na]^-$ (negative mode).

For **28**: $[\alpha]_D$ +15° (*c* 0.04, $H_2O$); $^1H$ NMR ($D_2O$, 293 K) δ 1.474 (d, 3H, CH*Me*CON, J = 6.4 Hz), 2.031 (s, 3H, NH*Ac*), 2.078 (NHNH*Ac*), 3.376 (dd, 1H, H-$2^d$), 3.565 (s, 3H, OMe), 3.930 (bs, 1H, H-$4^e$), 4.122 (bs, 1H, H-$4^c$), 4.153 (bs, 1H, H-$4^a$), 4.304 (d, 1H, H-$1^a$, $J_{1,2}$ = 8.6 Hz), 4.407 (q, 1H, C*H*MeCONHNH, J = 6.4 Hz), 4.465 (d, 1H, H-$1^e$, $J_{1,2}$ = 7.5 Hz), 4.573 (d, 2H, H-$1^c$ and H-$1^d$, $J_{1,2}$ = 7.8 Hz), 4.732 (d, 1H, H-$1^b$, $J_{1,2}$ = 8.1 Hz); $^{13}C$ NMR ($D_2O$) δ 18.37 (CH*Me*), 20.38 (*C*$H_3$CONHNH), 22.84 (*C*$H_3$CON), 55.90 (C-$2^b$), 57.87 (OMe), 103.05, 103.21, 103.46, 103.60, 104.57 (5 x C-1), 173.86 (MeCH*C*ONHNH), 175.63 (CH$_3$*C*ON and CH$_3$*C*ONHNH); FABMS for $C_{38}H_{65}N_3O_{28}$ (1011.9): *m/z* 1033.5 $[M-H+Na]^+$, 1011.7 $[M]^+$ (positive mode).



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