ELSEVIER

Carbohydrate Research 316 (1999) 121–132

CARBOHYDRATE RESEARCH

Synthesis of lacto-*N*-neotetraose and lacto-*N*-tetraose using the dimethylmaleoyl group as amino protective group

Mohamed R.E. Aly, El-Sayed I. Ibrahim [1], El-Sayed H. El Ashry [2], Richard R. Schmidt *

Fakultät Chemie, Universität Konstanz, M 725, D-78457 Konstanz, Germany

Received 9 November 1998; accepted 16 February 1999

Abstract

The disaccharide donor *O*-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-α,β-D-glucopyranosyl] trichloroacetimidate (**7**) was prepared by reacting *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl) trichloroacetimidate with *tert*-butyldimethylsilyl 3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleoylamido-glucopyranoside to give the corresponding disaccharide **5**. Deprotection of the anomeric center and then reaction with trichloroacetonitrile afforded **7**. Reaction of **7** with 3′-*O*-unprotected benzyl (2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**8**) as acceptor afforded the desired tetrasaccharide benzyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside. Replacement of the *N*-dimethylmaleoyl group by the acetyl group, O-debenzylation and finally O-deacetylation gave lacto-*N*-neotetraose. Similarly, reaction of *O*-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-dimethylmaleimido-α,β-D-glycopyranosyl] trichloroacetimidate as donor with **8** as acceptor afforded the desired tetrasaccharide benzyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-(4,6-benzylidene-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside. Removal of the benzylidene group, replacement of the *N*-dimethylmaleoyl group by the acetyl group and then O-acetylation afforded tetrasaccharide intermediate **15**, which carries only *O*-benzyl and *O*-acetyl protective groups. O-Debenzylation and *O*-deacetylation gave lacto-*N*-tetraose (**1**). Additionally, known *tert*-butyldimethylsilyl (2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside was transformed into *O*-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-dimethylmaleimido-α,β-D-glucopyranosyl] trichloroacetimidate as glycosyl donor, to afford with **8** as acceptor the corresponding tetrasaccharide **22**, which is transformed into **15**, thus giving an alternative approach to **1**.

Keywords: Lacto-*N*-tetraose; Lacto-*N*-neotetraose; Protecting groups, dimethylmaleoyl; Trichloroacetimidate; Glucosamine

1. Introduction

The tetrasaccharides lacto-*N*-tetraose (**1**) and lacto-*N*-neotetraose (**2**) are amongst the oligosaccharides isolated from human milk [1,2]; the former was the first aminodeoxy

* Corresponding author. Tel.: +49-7531-882538; fax: +49-7531-883135.

[1] Present address: Chemistry Department, Faculty of Science, Suez Canal University, Ismailia, Egypt.

[2] Present address: Chemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt.

0008-6215/99/$ - see front matter
PII: S0008-6215(99)00051-8

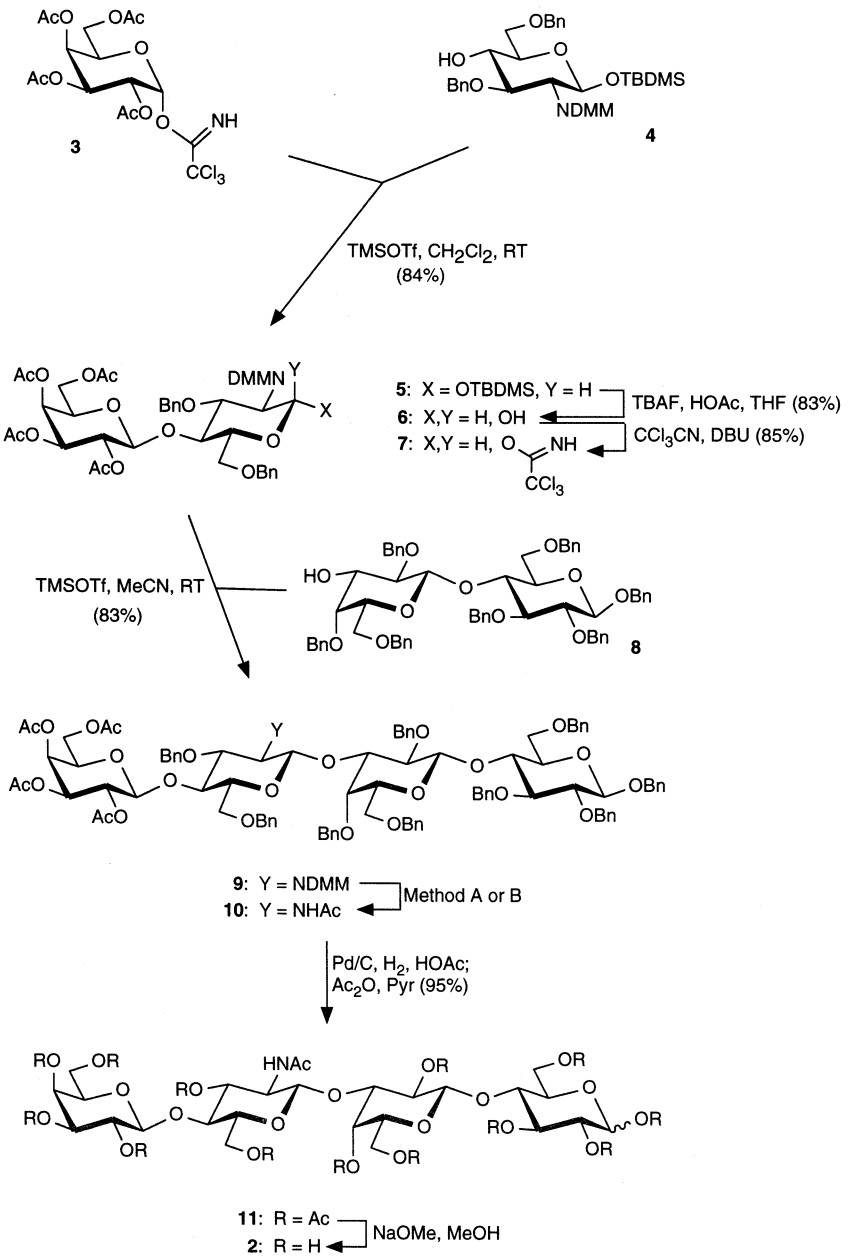
oligosaccharide that was found to occur free in nature [3–5]. Oligosaccharides **1** and **2** represent core structural elements of more complex oligosaccharides in human milk [1,2], in glycolipids, such as paragloboside and sialosylparagloboside, and in glycoproteins. These oligosaccharides are useful in the recognition of the acceptor specificity of glycosyltransferases, the substrate specificity of glycosidases, and the structure of antigenic determinants [1]. Thus, the sequence contained in lacto-*N*-neotetraose had been recognized by an antibody bound to granulocytes that was obtained by immunization with normal human granulocytes [6]. Paragloboside, a lactoneotetraosyl ceramide, was first isolated from human erythrocytes [7]; it was also identified as a specific surface component of virus polyoma-transformed hamster embryo fibroblast cells [8] and thus it may act as surface antigen associated with this tumor [9]. The Neu5Ac α-(2→6)-lacto-*N*-neotetraose was found to cause inhibition of the bacterial infection of a human patient suffering from pneumonia [10]. Both **1** and **2** act as bacterial receptors for pneumococci [11,12]. The pentasaccharide HSO_3-3Gal-β-(1→3)-GlcNAc-β-(1→3)-Gal-β-(1→4)Glc is one of the most potent oligosaccharide ligands for human E-selectin [13]. It is a strong supporter of the adhesion of E-selectin-expressing cells and an inhibitor of E-selectin adhesion. Such inhibitors may be used for the treatment of disorders of inflammation and for minimizing risk of metastasis after surgery reactions of tumors [13–15]. Partially protected lacto-*N*-neotetraoses were prepared as intermediates for the synthesis of blood group I-active biantennary neolacto-type decaosyl ceramide [16,17].

One of the crucial points in the syntheses of oligosaccharides incorporating 2-amino-2-deoxy-D-glucose necessitates its availability as a donor that requires a suitable protection of the amino group. Various protecting groups for such purposes are now available. Thus, the 2-deoxy-2-phthalimido sugars were used as glycosyl donors for the synthesis of lacto-*N*-neotetraose derivatives [16,17], of *p*-nitrophenyl lacto-*N*-tetraoside [18], and of benzyl lacto-*N*-neotetraoside [19]. Also lacto-*N*-biose was prepared by the phthalimide method [20,21]. The benzyl glycoside of **2** [22] as well as the ceramide of **1** [23] were prepared by using 2-azido-2-deoxy glucose as a precursor for the *N*-acetylglucosamine moiety. The methyl lacto-*N*-neotetraoside and derivatives thereof were prepared by the phthalimide method [24–26] and the oxazoline method [27,28]. The latter method was also used for the synthesis of lacto-*N*-tetraose by a route utilizing the oxazoline derivative of β-D-galactopyranosyl-(1→3)-D-glucosamine (lacto-*N*-biose I) as a donor for a suitably protected lactose acceptor [29–31]. Also the 2-*O*-methyl derivative of the galactose moiety at the nonreducing end has been prepared via the oxazoline method [32]. Recently, we introduced the dimethylmaleoyl (DMM) group as a new amino protecting group for glucosamine [33]. Its ease of attachment and subsequent cleavage as well as its electron-withdrawing property make the D-glucosamine derivative a good glycosyl donor with an enforced capability of forming a β-linkage. Moreover, versatile glycosyl acceptors containing the dimethylmaleoylamido group could also be generated and successfully employed in glycosylation reactions [33]. Owing to these perspectives of the DMM group, we report herein synthetic routes, using this group, to the biologically important lacto-*N*-neotetraose (**2**) and lacto-*N*-tetraose (**1**). The generated building blocks also allow their further use as donors or acceptors for the preparation of various oligosaccharides.

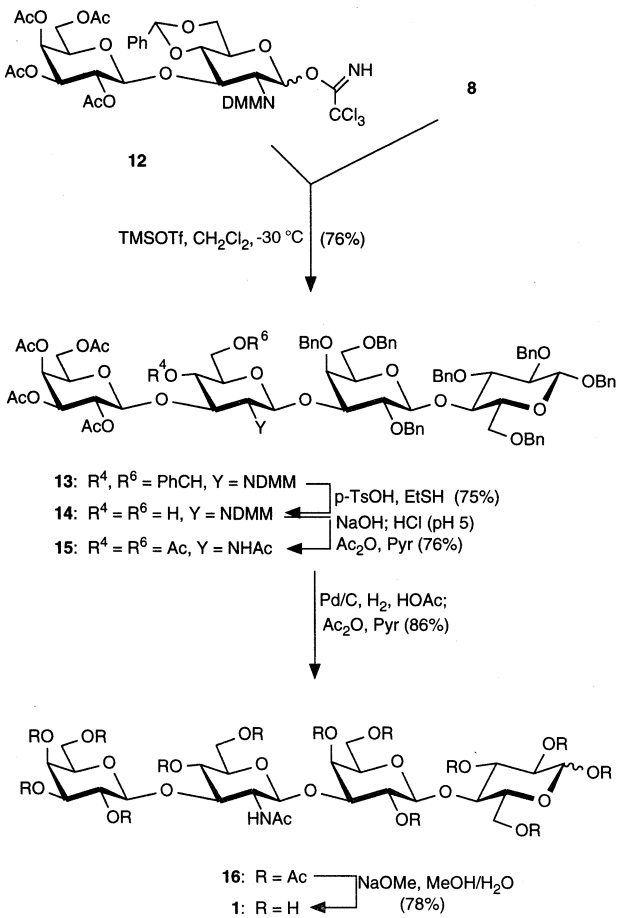
2. Results and discussion

For the synthesis of the target tetrasaccharides, final linkage of two disaccharides has been designed. Thus, the disaccharide donor will have the glucosamine residue containing the DMM group at the reducing end. The required disaccharide donor **7** was synthesized in three steps from known acceptor **4** [33] by its galactosidation with known trichloroacetimidate **3** [34] under the catalysis of trimethylsilyl trifluoromethanesulfonate (Me_3-SiOTf) to give the β-linked disaccharide **5** in 84% yield. Its ^{1}H NMR spectrum supported

the assignment; two doublets for H-1 and H-1′ at δ 5.10 and 4.61 with a J value of 8.0 Hz for each indicated the axial nature of H-1 and H-2 in both rings. The *tert*-butyldimethylsilyl (TBDMS) group was cleaved from **5** with tetrabutylammonium fluoride (TBAF) to give **6**, which can be readily transformed into the trichloroacetimidate donor **7**. Its reaction with the known disaccharide acceptor benzyl (2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**8**) [35] gave the desired tetrasaccharide **9**; the ^{13}C NMR spectrum showed the presence of four signals at δ 100.20, 100.70, 102.70, and 102.80 corresponding to the four β-linked anomeric carbons. Deblocking of the DMM group from **9** with NaOH to open the DMM ring followed by treatment with HCl to cleave the presumably formed butenolide and then intermediate acetylation gave **10** in 71% yield. On the other hand, using hydrazine hydrate as a deblocking agent followed by acetylation led to the formation of **10** in 54% yield. The success of this transformation was readily confirmed by ^1H NMR spectroscopy, which exhibited the disappear-

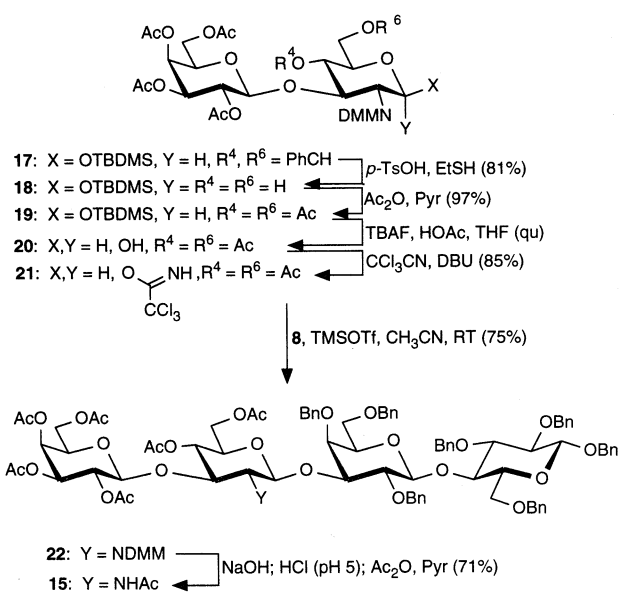


Scheme 1.



Scheme 2.

ance of the methyl signals of the dimethylmaleoyl group and the appearance of an NH signal at δ 5.8 and of a new methyl signal. Catalytic hydrogenation led to deblocking of the benzyl groups which, upon acetylation, gave **11** in 95% yield as the peracetyl derivative of **2**. The ^{1}H NMR spectrum of **11** indicated its presence as a mixture of α (δ 6.23) and β (δ 5.64) anomers in a ratio of 3:2. Almost the same ratio was also reported based on the calculation of specific rotations [28] and ^{1}H NMR spectroscopy [21]. Deacylation of **11** gave **2** in 91% yield. The ^{13}C NMR data of **2** were in accordance with published values [35]; reacetylation of **2** led again to compound **11**, thus confirming the structure of **2** (Scheme 1).



Scheme 3.

For the synthesis of the lacto-*N*-tetraose **1**, two approaches were designed. Both methods were based on the coupling of a disaccharide donor with the disaccharide acceptor **8** [36]. The designation of these two routes has been developed in order to allow for the presence of temporary blocking groups on the resulting tetrasaccharide thus making it suitable as acceptor for other mono- and oligosaccharide donors. In the first approach known donor **12** [33] was coupled with **8** to give **13** in 76% yield. Although its ^{1}H NMR spectrum showed a doublet at δ 5.27 for the newly formed glycosidic bond with a J value of 8.4 Hz confirming its β-configuration, the signals for the anomeric protons are hidden under other signals. However, their coupling with H-2 can be depicted from the signals for H-2. On the other hand, the ^{13}C NMR spectrum showed signals at δ_c 100.30, 100.50, and 102.80 corresponding to the four β-linked anomeric carbons. Debenzylidenation of **13** with *p*-TsOH gave **14** whose NMR spectra showed the absence of the benzylidene CH. The transformation of the DMM group to an acetyl group was achieved by the action of alkali and then acidification followed by acetylation to give **15** in 76% yield. Catalytic hydrogenation of **15**, followed by acetylation, gave the peracetyl derivative **16** as a mixture of α and β anomers in a ratio of 1:2, respectively (Scheme 2).

Alternatively, the required disaccharide donor **21** was prepared from known **17** [33] by debenzylidenation with *p*-TsOH to give **18** whose O-acetylation gave **19**. Subsequent removal of the TBDMS group with TBAF gave 1-O-unprotected **20**, thus being suitable for the activation with trichloroacetonitrile in the presence of DBU to give trichloroacetimidate

21. Coupling of **21** with **8** under standard conditions gave tetrasaccharide **22** in 75% yield. Its ^{1}H NMR spectrum showed for the H-1c a doublet at δ 5.16 with a coupling constant of 8.4 Hz, thus confirming the β-configuration. Moreover, its ^{13}C NMR spectrum showed signals at δ_c 99.90, 100.50, 102.8 (2C), confirming the presence of the four anomeric centers. Removal of the DMM group from **22** and subsequent acetylation afforded **15**, which was identical to the material obtained via the first approach (Scheme 3).

In conclusion, lacto-*N*-tetraose (**1**) and lacto-*N*-neotetraose (**2**) were successfully synthesized by the elaboration of the newly developed protection of amino sugars with dimethylmaleic anhydride providing the dimethylmaleimido group. Cleavage by addition of alkali followed by acid can be successfully performed as exhibited in our first report [33], using this group for amino sugars, and in other reports, using it for amino acids and proteins [37–41]. Its combination with the trichloroacetimidate leaving group [42] at C-1 of a glucosamine residue either in monomeric form or as part of an oligosaccharide provides good glycosyl donors in order to form β-linkages. Moreover, the DMM group tolerates manipulations of the common temporary protective groups required during oligosaccharide synthesis and, in addition, it can be selectively removed under quite mild conditions. The partially protected target tetrasaccharides prepared in this paper, as well as their precursors, can be used for the elaboration of various higher oligosaccharides.

3. Experimental

General methods.—Solvents were purified in the usual way. TLC was performed on plastic plates Silica Gel 60 F_{254} and on HPTLC plates NH_2 F_{254} S (E. Merck, layer thickness 0.2 mm). The detection was achieved by treatment with a solution of 20 g ammonium molybdate and 0.4 g cerium(IV) sulfate in 400 mL 10% H_2SO_4 or with 15% H_2SO_4, and heating at 150 °C. Flash chromatography was carried out on silica gel (Baker, 30–60 (μm) and Lichroprep NH_2, particle size 40–63 μm (E. Merck). Medium-pressure liquid chromatography (MPLC): LiChroprep Si 60 (E. Merck; size 15–25 μm), detection by differential refractometer. Optical rotations were determined at 25 °C with a Perkin–Elmer 241/MC polarimeter (1 dm cell). NMR spectra were recorded with Bruker AC 250 and 600 DRX instruments, using tetramethylsilane as internal standard. The assignments of ^{1}H NMR spectra were based on chemical shift correlation (DQFCOSY) and rotating frame nuclear Overhauser effect spectroscopy (ROESY). The assignments of ^{13}C NMR spectra were based on carbon–proton shift-correlation heteronuclear multiple quantum coherence (HMQC). MS spectra were recorded with MALDI-Kompakt (Kratos), EI and FAB with Finningen MAT 312/AMD. Microanalyses were performed in the Microanalysis Unit at the Fakultät für Chemie, Universität Konstanz.

tert-*Butyldimethylsilyl (2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)*-(1→4)-*3,6-di*-O-*benzyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranoside* (**5**).—A solution of **3** (0.25 g, 0.52 mmol) and **4** (0.24 g, 0.4 mmol) in dry CH_2Cl_2 (2 mL) was stirred under nitrogen at rt for 10 min while Me_5SiTf (0.01 M in CH_2Cl_2, 0.46 mL) was added dropwise. After 45 min, the reaction mixture was neutralized with Et_3N and evaporated under reduced pressure. The residue was purified by flash chromatography (2:1 petroleum EtOAc) to yield **5** (0.32 g, 84%) as an oil. TLC (2:1 petroleum EtOAc): R_f 0.34, $[\alpha]_D$ +19.8° (*c* 0.6, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.36–7.10 (m, 10 H, 2 Ph), 5.26 (d, 1 H, $J_{3,4}$ 2.9 Hz, H-4b), 5.13 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.4 Hz, H-2b), 5.10 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1a), 4.86 (dd, 1 H, H-3b), 4.61 (d, 1 H, H-1b), 4.73, 4.50 (2 d, 2 H, J_{gem} 12.2 Hz, CH_2Ph), 4.85, 4.41 (2 d, 2 H, J_{gem} 12.4 Hz, CH_2Ph), 4.12 (dd, 1 H, $J_{2,3}$ 10.8, $J_{3,4}$ 8.8 Hz, H-3a), 3.98–3.93 (m, 3 H, H-4a, H-6b, H-6′b), 3.84 (dd, 1 H, H-2a), 3.73 (dd, 1 H, J_{gem} 11.0, $J_{5,6'}$ 3.4 Hz, H-6′a), 3.68–3.65 (m, 2 H, H-5b, H-6a), 3.44 (m, 1 H, H-5a), 2.05, 1.99, 1.98, 1.95 (4 s, 12 H, 4 CH_3CO), 1.80 (br.s, 6 H, 2 CH_3), 0.72 [s, 9 H, $SiC(CH_3)_3$], 0.02 (s, 3 H, $SiCH_3$), −0.09 (s, 3

H, $SiCH_3$). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 170.31, 170.20, 170.03, 169.17 (4 CH_3CO, 2 CO), 139.10–136.69, 128.51–127.03 (2 Ph), 100.38 (C-1b), 93.43 (C-1a), 78.03 (C-4a), 74.76 (C-3a), 74.08 (C-5a), 73.58 (CH_2Ph), 71.03 (CH_2Ph), 70.48 (C-3b), 69.53 (C-5b), 67.72 (C-2b), 67.06 (C-6a), 66.95 (C-4b), 60.85 (C-6b), 57.54 (C-2a), 25.32 [$SiC(CH_3)_3$], 20.75, 20.64, 20.59, 20.54 (4 CH_3CO), 17.57 [$SiC(CH_3)$], 8.00 (2 CH_3), −4.24 ($SiCH_3$), −5.62 ($SiCH_3$). FABMS (positive mode, NBOH/NaI-matrix): m/z 934 [MNa^+]. Anal. Calcd for $C_{46}H_{61}NO_{16}Si$ (912.04): C, 60.57; H, 6.74; N, 1.53. Found: C, 60.17; H, 6.94; N, 1.50.

O-*[(2,3,4,6-Tetra*-O-*acetyl-β-*D*-galactopyranosyl)*-(1→4)-*3,6-di*-O-*benzyl-2-deoxy-2-dimethylmaleimido - α,β - D - glucopyranosyl trichloroacetimidate* (**7**).—A solution of **5** (3.0 g, 3.28 mmol) in dry THF (8 mL) in an ice-salt bath was treated with glacial AcOH (0.21 mL, 3.54 mmol) and TBAF (0.1 M, 3.6 mL, 3.6 mmol) with stirring. After 1 h, the ice bath was removed and the solution was stirred overnight then diluted with a saturated NaCl solution (20 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was separated, dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by flash chromatography (1.5:1 EtOAc–petroleum ether) to yield **6** (2.2 g, 83%) as a white foam. A mixture of **6** (2.2 g, 2.75 mmol), trichloroacetonitrile (0.6 mL, 5.8 mmol) and 1,8-diazabicyclo(5,4,0)undec-7-ene (0.02 mL, 0.12 mmol) in dry CH_2Cl_2 (6 mL) was stirred at rt for 8 h and then concentrated under reduced pressure. The residue was purified by flash chromatography (1:1 petroleum ether–EtOAc + 1% triethylamine) to yield **7** (2.23 g, 85%) as a pale yellow foam in the α:β ratio 1:3. TLC (1:1 petroleum ether–EtOAc + 1% triethylamine): R_f 0.5 (β-form) and R_f 0.6 (α-form); ^1H NMR (250 MHz, d_6-Me_2SO): δ 9.92 (br.s, 0.75 H, NH_β), 8.92 (s, 0.25 H, NH_α), 7.40–7.06 (m, 10 H, 2 Ph), 6.11 (d, 0.75 H, $J_{1,2}$ 8.5 Hz, H-1a_b), 5.84 (d, 0.25 H, $J_{1,2}$ 4.5 Hz, H-1a_a), 5.25 (d, 1 H, $J_{3,4}$ 2.7 Hz, H-4b), 5.10–3.41 (m, 16 H), 2.07, 2.00, 1.96, 1.92, 1.75 (5 s, 18 H, 2 CH_3, 4 $COCH_3$). FABMS (positive mode, NBOH/NaI-matrix): m/z 965 [MNa^+], 1115 [MNaI]Na^+. Anal. Calcd for $C_{42}H_{47}N_2O_{16}Cl_3$ (942.18): C, 53.53; H, 5.02; N, 2.97. Found: C, 53.18; H, 5.02; N, 3.09.

Benzyl (2,3,4,6-tetra-O-*acetyl-β-*D*-galactopyranosyl)*-(1→4)-*(3,6-di*-O-*benzyl-2-deoxy-2-dimethylmaleimido - β - D - glucopyranosyl)*-(1→3)-*(2,4,6-tri*-O-*benzyl-β-*D*-galactopyranosyl)*-(1→4)-*2,3,6-tri*-O-*benzyl-β-*D*-glucopyranoside* (**9**).—A mixture of **7** (0.414 g, 0.439 mmol) and **8** (0.333 g, 0.342 mmol) in dry MeCN (2 mL) was stirred under nitrogen at rt for 10 min while Me_3SiOTf (0.01 M in MeCN, 0.4 mL) was added dropwise. After 2 h, the solution was neutralized with triethylamine and evaporated under reduced pressure. The residue was purified by flash chromatography (2:1 petroleum ether–EtOAc) to yield **9** (0.501 g, 83%) as a white foam. TLC (2:1 petroleum ether–EtOAc): R_f 0.14; $[\alpha]_D$ + 11.2° (c 0.4, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 7.34–7.07 (m, 45 H, 9 Ph), 5.26 (d, 1 H, $J_{3,4}$ 3.0 Hz, H-4d), 5.21 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1c), 5.14 (dd, 1 H, $J_{1,2}$ 8.4, $J_{2,3}$ 10.2 Hz, H-2d), 5.04 (d, 1 H, J_{gem} 11.4 Hz, CH*H*Ph), 4.92 (d, 1 H, J_{gem} 10.5 Hz, C*H*HPh), 4.88–4.82 (m, 4 H, H-3d, 1.5 CH_2Ph), 4.69 (d, 1 H, J_{gem} 10.8 Hz, C*H*HPh), 4.66 (d, 1 H, J_{gem} 11.8 Hz, CH*H*Ph), 4.60–4.56 (m, 3 H, H-1d, CH_2Ph), 4.48 (d, 1 H, J_{gem} 11.4 Hz, C*H*HPh), 4.47 (d, 1 H, J_{gem} 12.1 Hz, C*H*HPh), 4.44 (d, 1 H, J_{gem} 11.8 Hz, C*H*HPh), 4.39–4.26 (m, 7 H, H-1b, H-1a, 2.5 CH_2Ph), 4.21–4.17 (m, 2 H, H-3c, C*H*HPh), 4.03–4.01 (m, 3 H, H-2c, H-4b, H-4c), 3.93 (m, 2 H, H-6d, H-6′d), 3.88 (m, 1 H, H-4a), 3.77–3.71 (m, 2 H, H-6c, H-6′c), 3.63 (m, 1 H, H-5d), 3.55–3.51 (m, 5 H, H-5c, H-6′b, H-2b, H-3b, H-6′a), 3.40–3.35 (m, 5 H, H-6b, H-6a, H-5b, H-2a, H-3a), 3.01 (m, 1 H, H-5a), 2.07, 1.99, 1.98, 1.97 (4 s, 12 H, 4 CH_3CO), 1.56 (br.s, 6 H, 2 CH_3). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 102.80 (C-1a), 102.70 (C-1b), 100.70 (C-1d), 100.20 (C-1c), 83.40 (C-3a), 82.50 (C-3b), 82.00 (C-2a), 79.30 (C-2b), 78.20 (C-4c), 77.30 (C-3c), 76.90 (C-4b), 76.40 (C-4a), 75.80 (CH_2Ph), 75.30 (2 CH_2Ph), 75.20 (C-5a), 74.70 (C-5, CH_2Ph), 74.60 (CH_2Ph), 74.20 (CH_2Ph), 73.80 (CH_2Ph), 73.50 (CH_2Ph), 73.30 (C-5b), 71.30 (CH_2Ph, C-3d), 70.80 (C-5d), 69.90 (C-2d), 68.60 (C-6b), 68.30 (C-6c), 68.20 (C-6a), 67.20 (C-4d), 60.90 (C-6d), 56.40 (C-2c). MALDI-

MS (positive mode, DHB/THF-matrix): *m/z* 1776 [MNa^+]. Anal. Calcd for $C_{101}H_{109}NO_{26}$ (1752.89): C, 69.20; H, 6.26; N, 0.79. Found: C, 69.26; H, 6.68; N, 0.78.

Benzyl (2,3,4,6-tetra-O-*acetyl-β*-D-*galacto-pyranosyl)*-(1→4)-*(2-acetamido-2-deoxy-3,6-di*-O-*benzyl-β*-D-*glucopyranosyl)*-(1→3)-*(2,4,-6-tri*-O-*benzyl-β*-D-*galactopyranosyl)*-(1→4)-*2,3,6-tri*-O-*benzyl-β*-D-*glucopyranoside* (**10**)

Method A. A mixture of **9** (0.542 g, 0.309 mmol) and NaOH (1.5 g, 0.037 mol) in MeOH–dioxane–water (5:2:1, 16 mL) was stirred overnight. Then, the solution was neutralized with 1:1 concd HCl–H_2O and the pH was adjusted to 5 by N HCl. After 24 h, the solution was neutralized with ethanolamine and dried well under reduced pressure. The residue was treated with pyridine–AcOH (2:1, 24 mL). After 20 h, the reaction mixture was co-evaporated with toluene under reduced pressure. The residue was diluted with water (30 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The organic layers were combined, dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by flash chromatography (1.5:1 petroleum ether–EtOAc) and the unreacted material was treated similarly to yield **10** (0.370 g, 71%) as a colorless oil. TLC (1.5:1 petroleum ether–EtOAc): R_f 0.09; $[\alpha]_D$ −6.2° (*c* 0.93, chloroform); ^1H NMR (600 MHz, $CDCl_3$): δ 7.34–7.17 (m, 45 H, 9 Ph), 5.80 (br.s, 1 H, NH), 5.28 (d, 1 H, $J_{3,4}$ 3.0, H-4d), 5.12 (dd, 1 H, $J_{1,2} = J_{2,3}$ 9.0 Hz, H-2d), 5.01 (d, 1 H, J_{gem} 11.6 Hz, C*H*HPh), 4.98 (d, 1 H, J_{gem} 10.6 Hz, C*H*HPh), 4.92–4.31 (m, 20 H, H-1b, H-1a, H-1d, H-3d, H-1c, 7.5 CH_2Ph), 4.18 (d, 1 H, J_{gem} 11.6 Hz, C*H*HPh), 4.01–3.90 (m, 5 H, H-6d, H-4a, H-4b, H-6′d, H-4c), 3.78–3.72 (m, 5 H, H-6′a, H-6c, H-6′c, H-3c, H-2c), 3.67–3.58 (m, 4 H, H-3b, H-5d, H-6a, H-2b), 3.52–3.49 (m, 3 H, H-3a, H-6′b, H-5c), 3.45–3.41 (m, 2 H, H-5b, H-2a), 3.35 (m, 1 H, H-6b), 3.24 (2 m, 1 H, H-5a), 2.01, 1.99, 1.98, 1.53 (4 s, 15 H, 5 CH_3CO). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 102.40 (C-1b), 102.30 (C-1a), 101.70 (C-1c), 99.80 (C-1d), 82.70 (C-3a), 81.70 (C-3b), 81.60 (C-2a), 79.70 (C-2b), 78.40 (C-3c), 76.30 (C-4c), 76.10 (C-4b), 75.80 (C-4a), 75.30 (*C*H_2Ph), 74.90 (C-5a), 74.80 (*C*H_2Ph), 74.70 (*C*H_2Ph), 74.60 (C-5c), 74.50 (*C*H_2Ph), 73.60 (*C*H_2Ph), 73.30 (*C*H_2Ph), 73.20 (*C*H_2Ph), 73.10 (*C*H_2Ph), 73.00 (C-5b), 70.80 (*C*H_2Ph), 70.60 (C-3d), 70.40 (C-5d), 69.30 (C-2d), 68.30 (C-6c), 68.10 (C-6b), 68.00 (C-6a), 66.70 (C-4d), 60.50 (C-6d), 55.10 (C-2c). MALDIMS (positive mode, DHB/THT-matrix): *m/z* 1711 [MNa^+]. Anal. Calcd for $C_{97}H_{107}N\,O_{25}$ (1686.83): C, 69.06; H, 6.39; N, 0.83. Found: C, 68.64; H, 6.55; N, 0.71.

Method B. A mixture of **9** (0.153 g, 0.087 mmol), hydrazine hydrate (3.096 g, 0.06 mol, 3.0 mL) in dry MeOH (6 mL) was heated under reflux. After 9 h, the solution was evaporated under reduced pressure and treated with Ac_2O and pyridine as described in *Method A* to yield **10** (0.08 g, 54%).

(2,3,4,6-Tetra-O-*acetyl-β*-D-*galactopyrano-syl)*-(1→4)-*(2-acetamido-2-deoxy-3,6-di*-O-*acetyl-β*-D-*glucopyranosyl)*-(1→3)-*(2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl)*-(1→4)-*1,2,3,-6-tetra*-O-*acetyl-α,β*-D-*glucopyranose* (**11**).—A solution of **10** (0.1 g, 0.059 mmol) in 1:1:1 AcOH–MeOH–dioxane (6 mL) was stirred under hydrogen in the presence of palladium on carbon (10% Pd, 0.1 g) at rt. After 48 h, the reaction mixture was filtered through Celite and washed with MeOH. The combined filtrates were concentrated under reduced pressure. The residue was treated with pyridine (10 mL), Ac_2O (5 mL) and stirred at rt. After 20 h, the reaction was worked up as described for **10**. The residue was purified by flash chromatography (2:1.5 toluene–acetone) to yield **11** (0.071 g, 95%) as an amorphous mass in the ratio of α/β 3:2. TLC (2:1.5 toluene–acetone): R_f 0.19; ^1H NMR (600 MHz, $CDCl_3$): δ 6.23 (d, 0.6 H, $J_{1,2} = J_{1,2} =$ 3.5 Hz, H-1a_α), 5.63 (d, 0.4 H, $J_{1,2}$ 8.3 Hz, H-1a_β), 5.4 (t, 0.6 Hz, $J_{2,3} = J_{3,4}$ 9.7 Hz, H-3a_α), 5.32 (m, 2 H, H-4d, NH), 5.28 (m, 1 H, $J_{3,4}$ 3.3 Hz, H-4b), 5.18–5.15 (m, 1.4 H, H-3c, H-3a_β), 5.8 (dd, 1 H, $J_{1,2} = J_{2,3}$ 8.2 Hz, H-2d), 5.02 (dd, 0.4 H, $J_{1,2}$ 8.3, $J_{2,3}$ 9.2 Hz, H-2a_β), 4.99–4.95 (m, 2.63 H, H-3d, H-2b, H-2a_α), 4.76 (m, 1 H, H-6′c), 4.65 (m, 1 H, H-1c), 4.52 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1d), 4.39–4.37 (m, 1 H, H-6′a_β, H-6′a_α), 4.31 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1b), 4.13–4.02 (m, 5 H, H-6b, H-6′b, H-6d, H-6′d, H-6a_β, H-6a_α), 3.96–3.94 (m, 1.6 H, H-6c, H-5a_α), 3.85 (br.t, 1 H, H-5d), 3.78–3.71 (m, 4.4 H, H-3b, H-5a_β, H-4a_α, H-5b,

H-4a$_\beta$, H-4c), 3.49–3.47 (m, 2 H, H-5c, H-2c), 2.16–1.88 (several s, 42 H, 14 CH_3CO). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 101.40 (C-1d), 101.10, 100.70 (C-1b), 100.50 (C-1c), 91.80 (C-1a$_\beta$), 89.20 (C-1a$_\alpha$), 76.00 (C-3b), 75.90 (C-4c), 75.40 (C-4a$_\alpha$), 75.30 (C-4a$_\beta$), 73.90 (C-5a$_\beta$), 73.00 (C-5c), 72.60 (C-3a$_\beta$), 72.10 (C-3c), 71.40 (C-5b), 71.30 (C-2a$_\alpha$), 71.10 (C-3d), 71.00 (C-5d), 70.70 (C-2a$_\beta$), 69.60 (C-3a$_\alpha$), 69.50 (C-2b), 69.40 (C-2d), 69.10 (C-4b), 66.90 (C-4d), 62.10 (C-6a$_\alpha$), 61.80 (C-6a$_\beta$, C-6b), 61.00 (C-6d), 60.50 (C-6c), 55.20 (C-2c). MALDIMS (positive mode, DHB/THT-matrix): m/z 1277 [MNa^+]. Anal. Calcd for $C_{52}H_{71}NO_{34}$ (1254.09): C, 49.79; H, 5.70; N, 1.11. Found: C, 49.81; H, 6.22; N, 1.15.

(β-D-Galactopysranosyl)(1→4)-*(2-acetamido-2-deoxy-β-D-glucopyranosyl)*(1→3)-*(β-D-galactopyranosyl)*-(1→4)-D-*glucopyranose* (**2**).—A mixture of **11** (0.041 g, 0.0326 mmol), MeOH (6.85 mL) and MeONa soln (0.195 M, 0.45 mL, 0.087 mmol) was stirred at rt. After 3 days, the mixture was neutralized with Amberlite IR 120 resin (H^+ form), filtered, evaporated under reduced pressure, and then lyophilized from water to yield **2** (0.021 g, 91%). TLC (1:1:0.5 CH_2Cl_2–MeOH–AcOH), R_f 0.08; HPTLC (4:1 EtOH–water), R_f 0.31. MALDIMS (positive mode, DHB/THF-matrix): m/z 732 [MNa^+]. Anal. Calcd for $C_{26}H_{45}NO_{21}\cdot 2H_2O$ (743.64): C, 41.99; H, 6.64; N, 1.88. Found: C, 41.94; H, 6.64; N, 1.68. The ^1H NMR spectroscopy data (600 MHz, D_2O) are in accordance with literature data [35].

Benzyl (2,3,4,6-tetra-O-*acetyl-β*-D-*galactopyranosyl)*-(1→3)-*(4,6-benzylidene-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl)*-(1→3)-*(2,4,6-tri*-O-*benzyl-β*-D-*galactopyranosyl)*-(1→4)-*2,3,6-tri*-O-*benzyl-β*-D-*glucopyranoside* (**13**).—A mixture of **12** (0.13 g, 0.15 mmol) and **8** (0.123 g, 0.126 mmol) in dry CH_2Cl_2 (1 mL) was stirred under nitrogen at −30 °C for 10 min while Me_3SiOTf (0.01 M in CH_2Cl_2, 0.17 mL) was added dropwise. After 40 min, the solution was neutralized with triethylamine and evaporated under reduced pressure. The residue was purified by flash chromatography (2:1 petroleum ether–EtOAc) to yield **13** (0.16 g, 76%) as a colorless oil. TLC (2:1 petroleum ether–EtOAc): R_f 0.12; $[\alpha]_D$ −8.6° (*c* 0.5, $CHCl_3$): ^1H NMR (600 MHz, $CDCl_3$): δ 7.40–7.10 (m, 40 H, 8 Ph), 5.57 (s, 1 H, C*H*Ph), 5.27 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1c), 5.22 (dd, 1 H, $J_{3,4}$ 2.6 Hz, H-4d), 5.00 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.3 Hz, H-2d), 4.94 (d, 1 H, J_{gem} 11.3 Hz, CH*H*Ph), 4.92 (d, 1 H, J_{gem} 10.5 Hz, CH*H*Ph), 4.86 (d, 1 H, J_{gem} 12.1 Hz, CH*H*Ph), 4.85 (d, 1 H, J_{gem} 10.8 Hz, C*H*HPh), 4.81 (dd, 1 H, $J_{3,4}$ 3.4 Hz, H-3d), 4.70 (d, 1 H, J_{gem} 10.8 Hz, C*H*HPh), 4.65 (dd, 1 H, $J_{2,3} = J_{3,4}$ 9.5 Hz, H-3c), 4.61 (d, 1 H, J_{gem} 10.5 Hz, C*H*HPh), 4.57 (d, 1 H, J_{gem} 10.5 Hz, C*H*HPH), 4.54–4.51 (m, 2 H, H-1d, C*H*HPh), 4.48 (m, 2 H, C*H*$_2$Ph), 4.39 (dd, 1 H, J_{gem} 10.5, $J_{5,6'}$ 4.8 Hz, H-6′c), 4.36–4.28 (m, 4 H, H-1a, H-1b, C*H*$_2$Ph), 4.27 (d, 1 H, J_{gem} 12.1 Hz, C*H*HPh), 4.22 (d, 1 H, J_{gem} 11.8 Hz, C*H*HPh), 4.14 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-2c), 4.05 (dd, 1 H, J_{gem} 11.0, $J_{5,6'}$ 8.1 Hz, H-6′d), 3.91–3.83 (m, 4 H, H-6c, H-6d, H-4b, H-4a), 3.77 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4c), 3.60–3.48 (m, 6 H, H-5d, H-6′b, H-6′a, H-2b, H-3b, H-5c), 3.41–3.35 (m, 5 H, H-6a, H-6b, H-5b, H-3a, H-2a), 2.98 (m, 1 H, H-5a), 2.09, 1.93, 1.90, 1.81 (4 s, 12 H, 4 CH_3CO), 1.55 (br.s, 6 H, 2 CH_3). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 102.80 (C-1a, C-1b), 101.90 (C-*C*HPh), 100.50 (C-1d), 100.30 (C-1c), 83.40 (C-3a), 82.10 (C-2a), 81.80 (C-3b), 81.20 (C-4c), 79.40 (C-2b), 76.80 (C-4b), 76.30 (C-4a), 75.70 (*C*H$_2$Ph), 75.40 (C-3c, *C*H$_2$Ph), 75.30 (*C*H$_2$Ph), 75.20 (C-5a), 74.50 (*C*H$_2$Ph), 73.80 (*C*H$_2$Ph), 73.50 (*C*H$_2$Ph), 73.40 (C-5b), 71.40 (C-3d), 71.20 (*C*H$_2$Ph), 70.70 (C-5d), 69.50 (C-2d), 69.10 (C-6c), 68.50 (C-6b), 68.10 (C-6a), 67.00 (C-4d), 66.30 (C-5c), 61.20 (C-6d), 56.00 (C-2c). MALDIMS (positive mode, DHB/THT-matrix): m/z 1684 [MNa^+]. Anal. Calcd for $C_{94}H_{101}NO_{26}$ (1660.75): C, 67.97; H, 6.13; N, 0.84. Found: C, 68.06; H, 6.41; N, 0.64.

Benzyl (2,3,4,6-tetra-O-*acetyl-β*-D-*galactopyranosyl)*-(1→3)-*(2-acetamido-4,6-di*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl)*-(1→3)-*(2,4,6-tri*-O-*benzyl-β*-D-*galactopyranosyl)*-(1→4)-*2,3,6-tri*-O-*benzyl-β*-D-*glucopyranoside* (**15**).—(a) From **13**. A mixture of **13** (0.165 g, 0.099 mmol), *p*-TsOH (0.005 g, 0.026 mmol) and ethanethiol (0.1 g, 1.62 mmol, 0.12 mL) in dry CH_2Cl_2 (1 mL) was stirred at rt. After 24 h, the solution was neutralized with Et_3N and evaporated under reduced pressure. The

residue was purified by flash chromatography (1:1 petroleum ether–EtOAc) to yield **14** (0.118 g, 75%) as an oil; TLC (1:1 petroleum ether–EtOAc): R_f 0.11. A mixture of the aforementioned oil (0.118 g, 0.075 mmol) and NaOH (0.17 g, 4.25 mmol) in a dioxane–water mixture (5:0.5, 5.5 mL) was stirred at rt. After 24 h, the pH of the solution was adjusted and kept at 5 by N HCl. After 24 h, the solution was neutralized with K_2CO_3 and dried under reduced pressure in the presence of ethanolamine (0.2 mL). The residue was treated with Ac_2O (4 mL) and pyridine (8 mL) and stirred at rt. After 15 h, the solution was worked up as described for **10**. The residue was purified by flash chromatography (1:1 petroleum ether–EtOAc) and the un-reacted material was separated and deprotected again to yield **15** (0.091 g, 76%) as a colorless oil. TLC (1:1 petroleum ether–EtOAc): R_f 0.09; $[\alpha]_D$ −12.0° (*c* 0.15, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.40–7.10 (m, 35 H, 7 Ph), 5.33 (br.s, 1 H, H-4d), 4.99–4.84 (m, 9 H, NH, H-4c, H-1c, H-3d, H-2d, 2.5 C*H*$_2$Ph), 4.73 (d, 1 H, J_{gem} 10.9 Hz, C*H*HPh), 4.69 (d, 1 H, J_{gem} 10.5 Hz, C*H*HPh), 4.63–4.57 (m, 3 H, 1.5 C*H*$_2$Ph), 4.50 (d, 1 H, J_{gem} 11.7 Hz, C*H*HPh), 4.44–4.40 (m, 3 H, H-1b, H-1a, C*H*HPh), 4.34–4.33 (m, 2 H, H-1d, C*H*HPh), 4.21–4.15 (m, 5 H, H-3c, H-6′d, H-6c, H-6′c, C*H*HPh), 4.07 (m, 1 H, H-6d), 3.98 (m, 1 H, H-4a), 3.92 (m, 1 H, H-4b), 3.83 (m, 1 H, H-5d), 3.76–3.74 (m, 2 H, H-5c, H-6′a), 3.67–3.66 (m, 2 H, H-6a, H-2b), 3.54–3.45 (m, 5 H, H-2c, H-2a, H-6′b, H-3a, H-3b), 3.40 (m, 1 H, H-5b), 3.36 (m, 1 H, H-6b), 3.28 (m, 1 H, H-5a), 2.13, 2.10, 2.05, 1.99, 1.95, 1.52 (6 s, 21 H, 7 CH_3CO). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 102.80 (C-1a, C-1b), 101.20 (C-1c), 101.10 (C-1d), 83.10 (C-3a), 82.10 (C-2a, C-3b), 80.70 (C-2b), 77.10 (C-3c), 76.70 (C-4a), 76.40 (C-4b), 75.50 (C-5a, *C*H$_2$Ph), 75.40 (*C*H$_2$Ph), 75.20 (*C*H$_2$Ph), 75.10 (*C*H$_2$Ph), 73.70 (2 *C*H$_2$Ph), 73.60 (C-5b), 72.10 (C-5c), 71.30 (C-3d, *C*H$_2$Ph), 70.80 (C-5d), 69.80 (C-4c), 69.40 (C-2d), 68.60 (C-6a), 68.40 (C-6b), 67.20 (C-4d), 63.00 (C-6c), 61.40 (C-6d), 57.40 (C-2c). FABMS (positive mode, NBOH/NaI-matrix): *m*/*z* 1613 [MNa$^+$]. Anal. Calcd for $C_{87}H_{99}NO_{27}$ (1590.66): C, 65.68; H, 6.27; N, 0.88. Found: C, 65.77; H, 6.58; N, 0.71.

(b) From **22.** Compound **22** (0.169 g, 0.102 mmol) was treated as described for **14** to yield **15** (0.116 g, 71%).

*(2,3,4,6-Tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1→3)-(2-acetamido-2-deoxy-4,6-di-*O*-acetyl-β-*D*-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-1,2,3,-6-tetra-*O*-acetyl-α/β-*D*-glucopyranose* (**16**).—According to the procedure described for the preparation of **11**, compound **15** (0.111 g, 0.07 mmol) was dissolved in 1:1:1 AcOH–MeOH–dioxane (4.5 mL) and hydrogenolized in the presence of Pd/C (10% Pd, 0.1 g) at rt for 48 h, then worked up and the residue was acetylated. The residue was purified by MPLC (1.5:1 toluene–acetone) to yield **16** (0.075 g, 86%) as an amorphous mass in the ratio of α:β 1:2. TLC (1.5:1 toluene–acetone): R_f 0.18; ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.23 (d, 0.33 H, $J_{1,2}$ 3.5 Hz, H-1a$_a$), 5.65–5.63 (m, 1.7 H, NH, H-1a$_\beta$), 5.41 (t, 0.3 H, $J_{2,3} = J_{3,4}$ 9.8 Hz, H-3a$_\alpha$), 5.32–5.31 (m, 2 H, H-4b, H-4d), 5.20 (t, 0.7 H, H-3a$_\beta$), 5.11 (t, 1 H, H-1c), 5.02–4.91 (m, 5 H, H-4c, H-3d, H-2a$_\alpha$, H-2b, H-2d, H-2a$_\beta$), 4.54 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.8 Hz, H-3c), 4.44–4.38 (m, 3 H, H-6′a$_\alpha$, H-6′a$_\beta$, H-6′c, H-1d), 4.33, 4.32 (2 d, 1 H, $J_{1,2}$ 7.8 Hz, H-1b), 4.14 (dd, 1 H, J_{gem} 12.1, $J_{5,6}$ 2.9 Hz, H-6a$_\alpha$, H-6a$_\beta$), 4.07–4.01 (m, 4 H, H-6b, H-6′b, H-6d, H-6′d), 3.98–3.96 (m, 1.3 H, H-5a$_\alpha$, H-6c), 3.82 (t, 1 H, H-5d), 3.77–3.73 (m, 3.7 H, H-3b, H-5a$_\beta$, H-5b, H-4a$_\alpha$, H-4a$_\beta$), 3.59 (br.d, 1 H, H-5c), 2.80 (br.t, 1 H, H-2c), 2.16–1.94 (several s, 42 H, 14 CH_3CO). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 101.30, 100.90 (C-1b), 101.00 (C-1d), 99.00 (C-1c), 91.90 (C-1aβ), 89.30 (C-1aα), 76.50 (C-3b), 76.30 (C-3c), 75.50 (C-4aα), 75.30 (C-4aβ), 74.00 (C-5aβ), 72.70 (C-3aβ), 72.20, 71.60 (C-5c), 71.40 (C-3d), 71.20 (C-2aα) 71.10 (C-5aα), 70.90 (C-5d), 70.70 (C-2aβ), 69.90 (C-2d), 69.70 (C-3aα, C-2b), 69.50 (C-4d), 69.20 (C-4c), 67.20 (C-4b), 62.00 (C-6a), 61.80 (C-6b), 61.70 (C-6c), 61.20 (C-6d), 59.30 (C-2c). MALDIMS (positive mode, DHB/THT-matrix): *m*/*z* 1278 [MNa$^+$]. Anal. Calcd for $C_{52}H_{71}NO_{34}$ (1254.09): C, 49.79; H, 5.70; N, 1.11. Found: C, 49.85; H, 6.23; N, 1.08.

*β-*D*-Galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1→3)-β-*D*-galactopyranosyl-(1→4)-*D*-glucopyranose* (**1**).

—A mixture of **16** (0.053 g, 0.033 mmol), MeOH (3.4 mL) and NaOMe (0.195 M in MeOH, 0.45 mL) was stirred at rt. After 3 h, water (3.4 mL) and NaOMe (0.195 M, 0.90 mL) were added. After 3 days, the reaction mixture was worked up as described for **2**. The residue was purified by flash chromatography on amino-phase Lichroprep (3:1 EtOH–water) to yield **1** (0.018 g, 78%) as a foam. HPTLC (4:1 EtOH–water): R_f 0.19; $[\alpha]_D + 23.9°$ (*c* 0.5, H_2O, 3 h); lit. $[\alpha]_D^{24} + 25.2°$ (final value) (*c* 1.5, H_2O) [5]. MALDIMS (positive mode, DHB/NaI-matrix): *m/z* 731.6 [MNa^+]. The ^1H NMR spectroscopy data (600 MHz, D_2O) were in accordance with literature data [35].

tert-*Butyldimethylsilyl(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)*-(1→3)-*2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranoside* (**18**). —A mixture of **17** (1.51 g, 1.84 mmol), *p*-TsOH (0.008 g, 0.42 mmol) and ethanethiol (0.84 g, 13.46 mmol, 1.0 mL) in dry CH_2Cl_2 (15 mL) was stirred at rt. After 20 h, the solution was neutralized with triethylamine and evaporated under reduced pressure. The residue was purified by flash chromatography (1:1 petroleum ether–EtOAc) to yield **18** (1.096 g, 81%) as a white foam. TLC (1:1 petroleum ether–EtOAc): R_f 0.14; $[\alpha]_D$ −6.4° (*c* 0.7, chloroform); ^1H NMR (600 MHz, $CDCl_3$): δ 5.31 (d, 1 H, $J_{3,4}$ 2.6 Hz, H-4b), 5.13 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.5 Hz, H-2b), 5.07 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1a), 4.91 (dd, 1 H, $J_{3,4}$ 2.6 Hz, H-3b), 4.35 (d, 1 H, H-1b), 4.32 (dd, 1 H, $J_{2,3}$ 10.8, $J_{3,4}$ 8.4 Hz, H-3a), 4.09 (m, 2 H, H-6b, H-6′b), 3.95 (m, 1 H, H-5b), 3.91–3.88 (m, 2 H, H-6′a, H-2a), 3.75 (dd, 1 H, J_{gem} 11.5, $J_{5,6}$ 5.6 Hz, H-6a), 3.54 (dd, 1 H, $J_{3,4}$ 8.4, $J_{4,5}$ 9.2 Hz, H-4a), 3.46 (m, 1 H, H-5a), 2.12, 2.05, 1.95, 1.92, 1.81 (5 s, 18 H, 2 CH_3, 4 CH_3CO), 0.73 [s, 9 H, $SiC(CH_3)_3$], 0.02 (s, 3 H $SiCH_3$), −0.07 (s, 3 H, $SiCH_3$). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 170.38, 170.10, 170.05, 168.68 (4 CH_3CO, 2 CO), 137.41 (2 C-DMM), 101.03 (C-1b), 93.41 (C-1a), 81.78 (C-3a), 75.36 (C-5a), 71.20 (C-5b), 70.76 (C-4a), 70.64 (C-3b), 68.60 (C-2b), 66.87 (C-4b), 63.17 (C-6a), 61.48 (C-6b), 56.87 (C-2a), 25.24 [$SiC(CH_3)_3$], 20.58, 20.53, 20.47, 20.27 (4 CH_3CO), 17.48 [$SiC(CH_3)$], 8.73 (2 C–CH_3), −4.10 ($SiCH_3$), −5.61 ($SiCH_3$). FABMS (positive mode, NBOH/NaI-matrix): *m/z* 754 [MNa^+]. Anal. Calcd for $C_{32}H_{49}NO_{16}Si$ (731.80): C, 52.51; H, 6.74; N, 1.91. Found: C, 52.63; H, 6.91; N, 1.77.

tert-*Butyldimethylsilyl(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)*-(1→3)-*4,6-di*-O-*acetyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranoside* (**19**).—A mixture of **18** (1.51 g, 1.43 mmol), pyridine (15 mL) and acetic anhydride (7.5 mL) was stirred at rt. After 18 h, the solution was worked up as described for **10**. The residue was purified by flash chromatography (1.5:1 petroleum ether–EtOAc) to yield **19** (1.141 g, 97%) as a white foam. TLC (1.5:1 petroleum ether–EtOAc: R_f 0.11; $[\alpha]_D$ −6.6° (*c* 0.5, $CHCl_3$), ^1H NMR (600 MHz, $CDCl_3$): δ 5.26 (d, 1 H, $J_{3,4}$ Hz, H-4b), 5.06 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1a), 4.93 (m, 1 H, H-2b), 4.92 (m, 1 H, H-4a), 4.84 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 3.4 Hz, H-3b), 4.54 (dd, 1 H, $J_{2,3}$ 10.8, $J_{3,4}$ 9.3 Hz, H-3a), 4.20 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1b), 4.13 (m, 2 H, H-6a, H-6′a), 4.07 (m, 2 H, H-6b, H-6′b), 3.95 (dd, 1 H, $J_{2,3}$ 10.8 Hz, H-2a), 3.76 (m, 1 H, H-5b), 3.70 (m, 1 H, H-5a), 2.10, 2.05, 2.04, 2.03, 2.02, 1.97, 1.91, 1.89 (8 s, 24 H, 2 CH_3, 6 CH_3CO), 0.73 [s, 9 H, $SiC(CH_3)_3$], 0.02 (s, 3 H, $SiCH_3$), −0.06 (s, 3 H, $SiCH_3$). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 170.32, 170.19, 169.29, 169.10 (6 CH_3CO, 2 CO), 100.37 (C-1b), 93.26 (C-1a), 75.08 (C-3a), 71.86 (C-5a), 70.84 (C-3b), 70.47 (C-5b), 69.70 (C-4a), 68.97 (C-2b), 66.76 (C-4b), 62.70 (C-6a), 60.76 (C-6b), 57.69 (C-2a), 25.27 [$SiC(CH_3)_3$], 20.78, 20.65, 20.53 (6 CH_3CO), 8.83 (2 CH_3), −4.24 ($SiCH_3$), −5.60 ($SiCH_3$). FABMS (positive mode, NBOH/NaI-matrix): *m/z* 838 [MNa^+], 988 [MNaI]Na^+. Anal. Calcd for $C_{36}H_{53}NO_{18}Si$ (815.87): C, 52.99; H, 6.54; N, 1.71. Found: C, 52.87; H, 6.73; N, 1.32.

O-*[(2,3,4,6-Tetra*-O-*acetyl-β*-D-*galactopyranosyl)*-(1→3)-*4,6-di*-O-*acetyl-2-deoxy-2-dimethylmaleimido-α/β*-D-*glucopyranosyl]trichloroacetimidate* (**21**).—A mixture of **19** (1.047 g, 1.28 mmol), glacial AcOH (0.084 g, 1.408 mmol, 85.0 μL) and TBAF (0.368 g, 1.41 mmol) in dry THF (10 mL) was reacted and worked up as described for **6**. The residue was purified by flash chromatography (2:1 EtOAc–petroleum ether) to yield **20** (0.9 g, quantitative) as a white foam. TLC (2:1

EtOAc–petroleum ether): R_f 0.24. A solution of the aforementioned foam (0.9 g, 1.28 mmol) in dry CH_2Cl_2 (5 mL) was treated by trichloroacetonitrile (0.83 mL, 8.1 mmol) and DBU (0.03 mL, 1.8 mmol) with stirring. After 9 h, the solution was evaporated under reduced pressure. The residue was purified by flash chromatography (1.5:1 EtOAc–petroleum ether + 1% Et_3N) to yield **21** (0.932 g, 85%) as a fluorescent foam in the ratio of α:β 1:5. TLC (1.5:1 EtOAc–petroleum ether + 1% Et_3N): R_f 0.28; ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.66 (s, 0.15 H, NH_α), 8.62 (s, 0.85 H, NH_β), 6.19 (d, 0.15 H, $J_{1,2}$ 3.2 Hz, H-1a_a), 6.15 (d, 0.85 H, $J_{1,2}$ 8.9 Hz, H-1a_β), 5.31–3.76 (m, 13 H), 2.13, 2.11, 2.08, 2.07, 1.98, 1.94, 1.81 (7 s, 24 H, 2 CH_3, 6 CH_3CO). FABMS (positive mode, NBOH/NaI-matrix): m/z 869 [MNa^+], 1019 [MNaI]Na^+. Anal. Calcd for $C_{32}H_{39}N_2Cl_3O_{18}$ (846.02): C, 45.42; H, 4.64; N, 3.31. Found: C, 44.86; H, 4.49; N, 3.43.

Benzyl (2,3,4,6-tetra-O-*acetyl-β*-D-*galacto-pyranosyl)*-(1→3)-*(4,6-di*-O-*acetyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranosyl)*-(1→3)-*(2,4,6-tri*-O-*benzyl-β*-D-*galactopyranosyl)*-(1→4)-*2,3,6-tri*-O-*benzyl-β*-D-*glucopyranoside* (**22**).—A mixture of **21** (0.16 g, 0.177 mmol) and **8** (0.15 g, 0.154 mmol) in dry MeCN (1 mL) was stirred under nitrogen at rt for 10 min while Me_3SiOTf (0.01 M in MeCN, 0.2 mL) was added dropwise and the mixture was stirred overnight, then neutralized with Et_3N and evaporated under reduced pressure. The residue was purified by flash chromatography (1.5:1 petroleum ether–EtOAc) to yield **22** (0.192 g, 75%) as a white foam. TLC (1.5:1 petroleum ether–EtOAc): R_f 0.13; $[\alpha]_D$ −12.3° (c 0.33, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.30–7.10 (m, 35 H, 7 Ph), 5.27 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4d), 5.16 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1c), 4.97 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4c), 4.94–4.91 (m, 3 H, H-2d, CH_2Ph), 4.86, 4.27 (2 d, 2 H, J_{gem} 12.1 Hz, CH_2Ph), 4.78 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 3.5 Hz, H-3d), 4.85, 4.70 (2 d, 2 H, J_{gem} 10.8 Hz, CH_2Ph), 4.62–4.51 (m, 5 H, H-3c, 2 CH_2Ph), 4.47 (d, 1 H, J_{gem} 11.5 Hz, 1 H, CHHPh), 4.36–4.31 (m, 4 H, H-1b, H-1a, CH_2Ph), 4.21–4.18 (m, 3 H, H-6c, H-6′c, CHHPh), 4.11–4.08 (m, 4 H, H-1d, H-2c, H-6d, H-6′d), 3.96 (dd, 1 H, $J_{3,4}$ 2.6 Hz, H-4b), 3.91 (m, 1 H, H-4a), 3.75 (m, 1 H, H-5c), 3.71 (m, 1 H, H-5d), 3.60–3.50 (m, 4 H, H-6′b, H-3b, H-2b, H-6′a), 3.41–3.36 (m, 5 H, H-6b, H-5b, H-6a, H-2a, H-3a), 2.99 (m, 1 H, H-5a), 2.10–1.90 (6 s, 18 H, 6 CH_3CO), 1.70 (br.s, 3 H, CH_3), 1.50 (br.s, 3 H, CH_3). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 102.80 (C-1a, C-1b), 100.50 (C-1d), 99.90 (C-1c), 83.30 (C-3a), 82.60 (C-3b), 82.00 (C-2a), 79.00 (C-2b), 76.70 (C-4b), 76.30 (C-4a), 75.70 (CH$_2$Ph), 74.50 (CH$_2$Ph), 75.20 (C-5a, CH$_2$Ph), 74.60 (C-3c), 74.30 (CH$_2$Ph), 73.70 (CH$_2$Ph), 73.60 (CH$_2$Ph), 73.40 (C-5b), 71.90 (C-5c), 71.10 (C-3d, CH$_2$Ph), 70.80 (C-5d), 69.70 (C-4c), 69.30 (C-2d), 68.50 (C-6b), 68.10 (C-6a), 67.00 (C-4d), 62.80 (C-6c), 60.90 (C-6d), 56.20 (C-2c). MALDIMS (positive mode, DHB/THT-matrix): m/z 1679 [MNa^+]. Anal. Calcd for $C_{91}H_{101}NO_{28}$ (1656.72): C, 65.96; H, 6.14; N, 0.84. Found: C, 65.55; H, 5.96; N, 0.78.

Acknowledgements

This work was supported by the European Community (grant No. ERB 4061 PL 95-0372), the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. M.R.E.A. is grateful for a stipend within the channel program of the Egyptian Government. The help of Dr Armin Geyer in structural assignments is gratefully acknowledged.

References

[1] A. Kobata, *Methods Enzymol.*, 28 (1972) 262–271.
[2] A. Kobata, K. Yamashita, Y. Tachibana, *Methods Enzymol.*, 50 (1978) 216–220.
[3] R. Kuhn, A. Gauhe, H.H. Baer, *Chem. Ber.*, 86 (1953) 827–831.
[4] R. Kuhn, A. Gauhe, H.H. Baer, *Chem. Ber.*, 87 (1954) 289–300.
[5] R. Kuhn, H.H. Baer, *Chem. Ber.*, 89 (1956) 504–511.
[6] S.L. Spitalik, J.F. Schwartz, J.L. Magnani, D.D. Roberts, P.F. Spitalnik, C.I. Civin, V. Ginsburg, *Blood*, 66 (1985) 319–326.
[7] (a) B. Siddiqui, S. Hakomori, *Biochem. Biophys. Acta*, 330 (1973) 147–155. (b) S. Ando, T. Yamakawa, *J. Biochem. (Tokyo)*, 73 (1973) 387–396.
[8] C.G. Gahmberg, S. Hakomori, *J. Biol. Chem.*, 250 (1975) 2438–2446.
[9] J.S. Sundsmo, S. Hakomori, *Biochem. Biophys. Res. Commun.*, 68 (1976) 799–806.

[10] P.M. Simon, D. Zopf, R.A. Barthelson, K.F. Johnson, *PCT Int. Appl. WO 96*, 40, 169 (1996); *Chem. Abstr.*, 126 (1997) 135643c.
[11] S. Hakomori, *Ann. Rev. Biochem.*, 50 (1981) 733–765.
[12] C. Svansborg-Edén, B. Andersson, L. Hagberg, H. Leffler, G. Magnusson, G. Noori, J. Dahém, T. Söderström, *Ann. N.Y. Acad. Sci.*, 409 (1983) 580–592.
[13] C.-T. Yuen, K. Benzouska, J. O'Brien, M. Stoll, R. Lemoine, A. Lubineau, M. Kiso, A. Hasegawa, N.J. Bockovick, K.C. Nicolaou, T. Feizi, *J. Biol. Chem.*, 269 (1994) 1595–1598.
[14] M.P. Bevilacqua, R.M. Nelson, *J. Clin. Invest.*, 91 (1993) 379–387.
[15] S.M. Edgington, *Bio/Technology*, 10 (1992) 383–389.
[16] (a) Y. Matsuzaki, Y. Ito, T. Ogawa, *Tetrahedron Lett.*, 33 (1992) 6343–6346. (b) Y. Matsuzaki, Y. Ito, T. Ogawa, *Tetrahedron Lett.*, 33 (1992) 4025–4028. (c) I. Chiu, V. Verez, D.S. Tleugabulova, M. Hernandez, C.S. Perez, *Rev. Cubana Quim.*, 6 (1992) 1–6; *Chem. Abstr.*, 120 (1994) 299101b.
[17] M. Bröder, H. Kunz, *Carbohydr. Res.*, 249 (1993) 221–241; *Bioorg. Med. Chem.*, 5 (1997) 1–19.
[18] R.K. Jain, R.D. Locke, K.L. Matta, *Carbohydr. Res.*, 241 (1993) 165–176.
[19] M.M. Ponpipom, R.L. Bugianesi, T.Y. Shen, *Tetrahedron Lett.*, (1978) 1717–1720.
[20] R.U. Lemieux, S.Z. Abbas, B.Y. Chung, *Can. J. Chem.*, 60 (1982) 58–62.
[21] R.U. Lemieux, S.Z. Abbas, B.Y. Chung, *Can. J. Chem.*, 60 (1982) 68–75.
[22] H. Paulsen, K.-M. Steiger, *Carbohydr. Res.*, 169 (1987) 105–125.
[23] R. Bommer, R.R. Schmidt, *Liebigs Ann. Chem.*, (1989) 1107–1111.
[24] A. Maranduba, A. Veyrières, *Carbohydr. Res.*, 135 (1985) 330–336.
[25] A. Maranduba, A. Veyrières, *Carbohydr. Res.*, 151 (1986) 105–119.
[26] W. Zou, H.J. Jennings, *J. Carbohydr. Chem.*, 15 (1996) 279–295.
[27] J. Dahmen, G. Gnosspelius, A.-C. Larsson, T. Lave, G. Noori, K. Palsson, *Carbohydr. Res.*, 138 (1985) 17–28.
[28] R.I. El-Sokkary, R.F. Bassily, R.H. Youssef, M.A. Nashed, *J. Carbohydr. Chem.*, 17 (1998) 267–268.
[29] T. Takamura, T. Chiba, H. Ishihara, S. Tejima, *Chem. Pharm. Bull.*, 27 (1979) 1497–1499.
[30] T. Takamura, T. Chiba, H. Ishihara, S. Tejima, *Chem. Pharm. Bull.*, 28 (1980) 1804–1809.
[31] C. Augé, A. Veyrières, *J. Chem. Soc., Perkin Trans. 1*, (1977) 1343–1345.
[32] A.K. Sarkar, R.K. Jain, K.L. Matta, *Carbohydr. Res.*, 203 (1990) 33–46.
[33] M.R.E. Aly, J.C. Castro-Palomino, E.I. Ibrahim, E.S.H. El Ashry, R.R. Schmidt, *Eur. J. Org. Chem.*, (1998) 2305–2316.
[34] R.R. Schmidt, J. Michel, M. Roos, *Liebigs Ann. Chem.*, (1984) 1343–1357.
[35] G. Strecker, J.-M. Wieruszeski, J.-C. Michalski, J. Montreuil, *Glycoconjugate J.*, 6 (1989) 67–83.
[36] K.-H. Jung, M. Hoch, and R.R. Schmidt, *Liebigs Ann. Chem.*, (1989) 1099–1106.
[37] H.B.F. Dixon, R.N. Perham, *Biochem. J.*, 109 (1968) 312–314.
[38] A.J. Kirby, P.W. Lancaster, *J. Chem. Soc., Perkin Trans. 2*, (1972) 1206–1214.
[39] A.J. Kirby, *Acc. Chem. Res.*, 30 (1997) 290–296.
[40] F. Wieland, L. Renner, C. Vorfürth, F. Lynen, *Eur. J. Biochem.*, 94 (1979) 189–197.
[41] T. Hirano, T. Todoroki, S. Kato, H. Yamamoto, P. Caliceti, F. Veronese, H. Maeda, S. Ohashi, *J. Controlled Release*, 28 (1994) 203–209.
[42] R.R. Schmidt, *Angew. Chem.*, 98 (1986) 213–236; *Angew. Chem., Int. Ed. Engl.*, 25 (1986) 212–235.