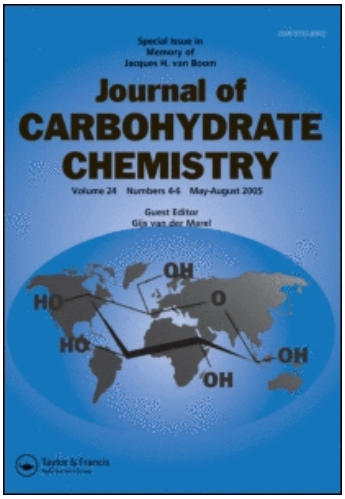

Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

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To cite this Article Park, So Mi , Suk, Dae-Hwan and Kim, Kwan Soo(2009) 'Stereoselective Synthesis of the Hexasaccharide Repeating Unit of the Cell Wall Polysaccharide from *Kineosporia aurantiaca* VKM Ac-720^T Employing the Direct Glycosylation with Anomeric Hydroxy Sugars Involving Glycosyl Phthalate Intermediates', Journal of Carbohydrate Chemistry, 28: 6, 317 — 329
To link to this Article: DOI: 10.1080/07328300903003352
URL: http://dx.doi.org/10.1080/07328300903003352

Journal of Carbohydrate Chemistry, 28:317–329, 2009

ISSN: 0732-8303 print / 1532-2327 online
DOI: 10.1080/07328300903003352

Stereoselective Synthesis of the Hexasaccharide Repeating Unit of the Cell Wall Polysaccharide from *Kineosporia aurantiaca* VKM Ac-720^T Employing the Direct Glycosylation with Anomeric Hydroxy Sugars Involving Glycosyl Phthalate Intermediates

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Synthesis of a suitably protected form of the hexasaccharide repeating unit of the cell wall polymer from *Kineosporia aurantiaca* VKM Ac-720^T has been achieved by the stereoselective direct glycosylation of a trisaccharide acceptor with a trisaccharide donor having an anomeric hydroxy group involving a glycosyl phthalate intermediate. Both the trisaccharide acceptor and the trisaccharide donor were obtained from a common trisaccharide, of which two β-mannopyranosyl linkages were constructed stereoselectively by employing the direct glycosylation method with the anomeric hydroxy sugar involving a glycosyl phthalate intermediate and the 2′-carboxybenzyl glycoside method, respectively.

Keywords Oligosaccharides; Glycosylation; 2′-Carboxybenzyl (CB) glycosides; Glycosyl phthalates; β-Mannosylation

Received March 11, 2009; accepted April 28, 2009.
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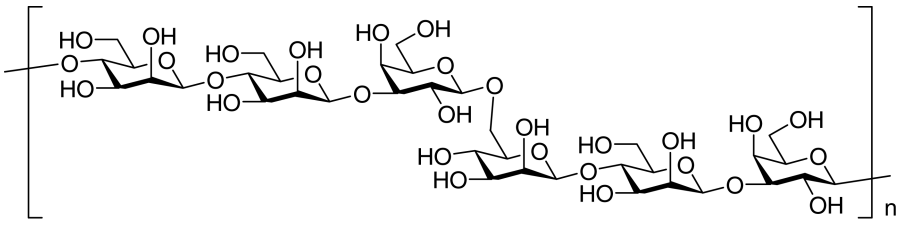
INTRODUCTION

Kineosporia, which is an aerobic gram-positive bacterium, was isolated from soil and classified as a genus of the order *Actinomycetales*.[1] Although it was originally proposed that the genus *Kineosporia* was constituted by a single species *Kineosporia aurantiaca*,[1] further studies amended the description of the genus[2] and added new species in the genus.[3] Extensive structural studies of the cell wall of *Actinomycetales* have been performed because the structure of cell wall polysaccharides and teichoic acids was known to be used as taxonomic makers or fingerprints of the actinomycete species.[4] Recently, Tul'skaya and coworkers have reported that the major cell wall polymer of *K. aurantiaca* VKM Ac-720^T is a neutral polysaccharide, a galactomannan with a previously unknown structure.[5] This particular galactomannan is composed of a series of the repeating unit of a hexasaccharide with the following structure:→4)-β-D-Man*p*-(1→4)-β-D-Man*p*-(1→3)-β-D-Gal*p*-(1→6)-β-D-Man*p*-(1→4)-β-D-Man*p*-(1→3)-β-D-Gal*p*-(1→(**A**), as shown in Figure 1. This is quite unusual because the major component of the cell wall polymer of *Actinomycetales* is not usually neutral polysaccharides but rather anionic polysaccharides.[4] An interesting structural feature of the hexasaccharide repeating unit of this galactomannan is the occurrence of the β-D-mannopyranosyl linkage, of which the stereospecific formation still poses a challenge. Several diverse and innovative strategies for the β-D-mannopyranosylation have been developed,[6] and recently Crich and coworkers have made a significant breakthrough in the β-mannoside synthesis employing 4,6-*O*-benzylidene-protected mannopyranosyl sulfoxides or thiomannopyranosides as glycosyl donors.[7] We have also reported successful 4,6-*O*-benzylidene-directed β-mannopyranosylations with our new glycosyl donors, anomeric hydroxy sugars involving glycosyl phthalate intermediates[8] and 2′-carboxybenzyl (CB) glycosides.[9]

Herein, as a part of our works on the synthesis of oligosaccharides originated from the cell wall of microorganisms[10] in the effort to achieve synthetically challengeable goals and/or to study the biological activity related to them, we present the stereoselective synthesis of hexasaccharide **1** as shown in



A

Figure 1: The repeating unit of the cell wall polysaccharide from *K. aurantiaca* VKM Ac-720^T.

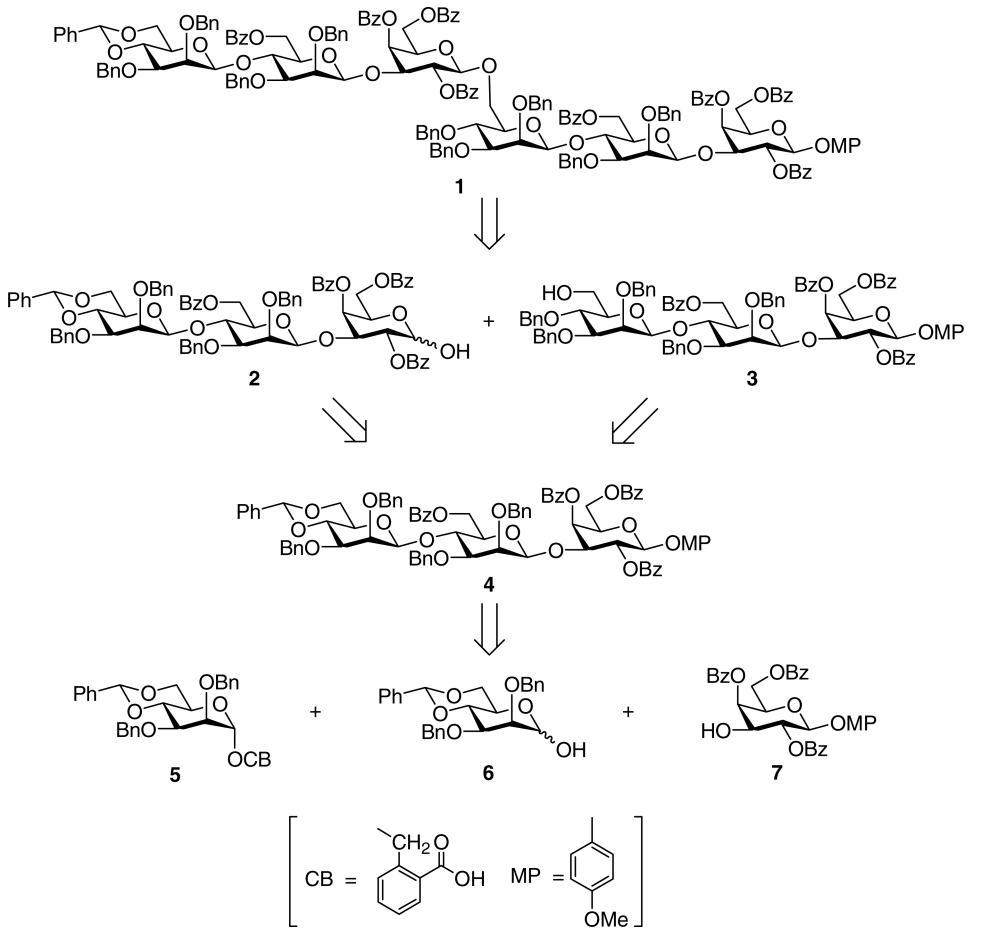


Figure 2: Retrosynthesis of hexasaccharide **1**.

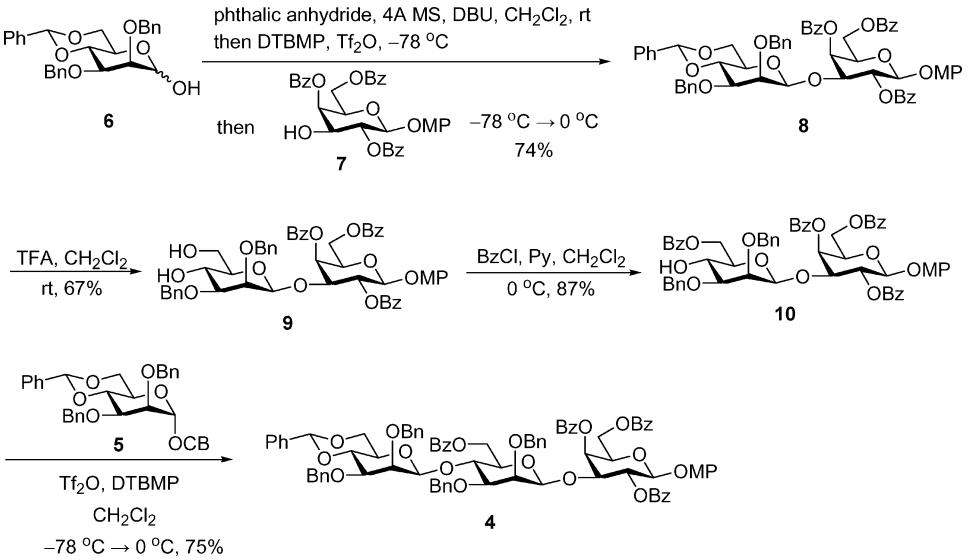
Figure 2, a suitably protected form of the hexasaccharide repeating unit **A** of the cell wall polymer from *K. aurantiaca* VKM Ac-720^T employing our newly developed glycosylation methods such as the direct glycosylation with anomeric hydroxy sugars involving glycosyl phthalate intermediates[8] and the CB glycoside method.[9]

RESULTS AND DISCUSSION

Protective groups of the target hexasaccharide **1** were selected with careful deliberation for the future synthesis of a dodecasaccharide or an octadecasaccharide by dimerization or trimerization of **1**. Thus, the 4,6-*O*-benzylidene group in the nonreducing end of **1** would be selectively cleaved to give a hexasaccharide acceptor with a hydroxy group at C-4. The methoxyphenyl (MP) group at

C-1 in the reducing end of **1** would be readily converted into a hexasaccharide having an anomeric hydroxy group as a donor. Retrosynthesis of the hexasaccharide **1** led to trisaccharide donor **2** and acceptor **3**, which both would be obtained from same trisaccharide **4** as shown in Figure 2. This common intermediate **4** was further analyzed into monosaccharide building blocks **5**, **6,** and **7**.

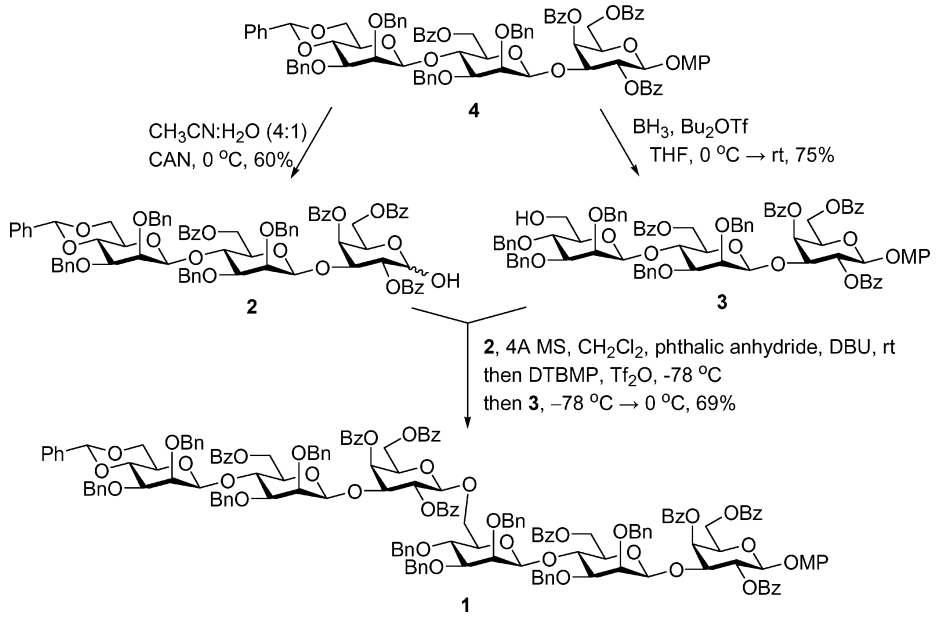
Our synthesis started with preparation of the monosaccharide building blocks **5**, **6**, and **7**. Thus, CB 4,6-*O*-benzylidenemannoside **5**,[9] 4,6-*O*-benzylidenemannose **6**,[11] and *p*-methoxyphenyl galactoside **7**[12] were synthesized according to previously reported procedures. Coupling of **6** and **7** was performed by the stereoselective β-mannopyranosylation of the acceptor **7** with the 4,6-*O*-benzyliden-protected anomeric hydroxy sugar **6** employing our new direct glycosylation method involving the glycosyl phthalate intermediate.[8] Thus, treatment of the mannose **6** with phthalic anhydride and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in methylene chloride at rt provided a mannosyl phthalate intermediate. Without isolation of the mannosyl phthalate intermediate, triflic anhydride and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) were added to the above solution at -78°C. Then, addition of the acceptor alcohol **7** to this solution provided exclusively β-mannosyl disaccharide **8** in 74% yield after chromatographic separation as shown in Scheme 1. The LC-mass analysis indicated that the β/α ratio of the crude product **8** was 30:1. Anomeric carbon chemical shifts at δ 101.6 and 101.1 ppm and one-bond coupling constants (J_{C1-H1}) of 165 and 162 Hz of the product **8** indicated that two glycosyl linkages including the newly generated mannosyl linkage,



Scheme 1.

D-Man*p*-(1→3)-D-Gal*p*, of the disaccharide **8** are both in β-configurations.[13] Hydrolysis of the 4,6-*O*-benzylidene group of the disaccharide **8** with trifluoroacetic acid (TFA) followed by selective benzoylation of the primary hydroxy group in resulting disaccharide diol **9** with benzoyl chloride at 0°C afforded 6-*O*-benzoyl disaccharide acceptor **10** having a free hydroxy group at C-4″. The β-mannosylation of the disaccharide acceptor **10** to make the mannosyl trisaccharide **4** was preformed with CB 4,6-*O*-benzylidenemannoside **5** as the mannosyl donor. Thus, the coupling of the CB glycosyl donor **5** and the glycosyl acceptor **10** in the presence of triflic anhydride and DTBMP produced predominantly the β-mannosyl trisaccharide **4** ($\beta/\alpha = 9{:}1$) in 75% yield. Other 4,6-*O*-benzylidene-protected mannosyl donors such as the corresponding mannosyl trichloroacetimidate[14] and mannose **6** involving a mannosyl phthalate intermediate[8] were found to be less satisfactory than the CB 4,6-*O*-benzylidene mannoside donor **5** in the case of the β-mannosylation of **10** to make **4**. Three one-bond coupling constants (J_{C1-H1}), 157.2, 159.9, and 161.9 Hz, again indicated that all glycosyl linkages including the newly generated one in the trisaccharide **4** are in β-configurations. A similar strategy for the construction of two consecutive β-mannosyl linkages, namely, β-(1→4) and β-(1→3) linkages, employing 4,6-*O*-benzylidene mannosyl donors have been reported for the synthesis of a mannan.[15]

Reductive cleavage of the 4,6-*O*-benzylidene group of the fully protected trisaccharide **4** with borane in the presence of Bu_2BOTf[16] afforded trisaccharide acceptor **3** having a free hydroxy group at C-6‴ as shown in Scheme 2.



Scheme 2.

Oxidative cleavage of the anomeric *p*-methoxyphenyl group of **4** with ceric ammonium nitrate (CAN) in CH_3CN/H_2O,[17] on the other hand, provided trisaccharide donor **2** having an anomeric hydroxy group at C-1. Final coupling of two trisaccharides **2** and **3** was accomplished by glycosylation of the acceptor **3** with the anomeric hydroxy sugar **2** as the donor using phthalic anhydride and Tf_2O as activators.[8] Thus, reaction of the trisaccharide donor **2** and phthalic anhydride in the presence of DBU at rt followed by sequential addition of DTBMP, triflic anhydride, and the trisaccharide acceptor **3** to the solution at $-78^\circ C$ produced predominantly desired hexasaccharide **1** ($\beta/\alpha = 10{:}1$) in 69% yield. Although synthesis of **1** by glycosylation of **3** with the glycosyl trichloroacetimidate derived from **2** also gave the desired hexasaccharide **1**, side products generated during the glycosylation made purification of **1** more difficult. Six one-bond coupling constants (J_{C1-H1}), 155, 160, 157, 160, 155, and 158 Hz, clearly indicated that all six glycosyl linkages including the newly generated one in the hexasaccharide **1** are in β-configurations.

In summary, synthesis of hexasaccharide **1**, a suitably protected form of the repeating unit of the cell wall polymer from *K. aurantiaca* VKM Ac-720^T, has been achieved by coupling of two trisaccharides **2** and **3**, both of which were obtained from common trisaccharide **4**. Direct one-pot glycosylation with anomeric hydroxy sugars involving glycosyl phthalate intermediates was employed for the stereoselective construction of the β-galactosyl linkage of hexasaccharide **1** and one of the β-mannosyl linkages of the trisaccharide **4** as key steps. Another β-mannosyl linkage of **4** was constructed by employing the CB glycoside method.

ACKNOWLEDGEMENT

This work was supported by a grant from the Korea Science and Engineering Foundation through the Center for Bioactive Molecule Hybrids (CBMH). S.M.P. and D.-H.S. thank the fellowship of the BK 21 program from the Ministry of Education and Human Resources Development.

EXPERIMENTAL

All reactions were conducted under a positive pressure of dry argon with dry, freshly distilled solvents unless otherwise noted. All reagents were purchased from commercial suppliers and used without further purification unless otherwise noted. Optical rotations were determined at $20^\circ C$ with an automatic polarimeter. ^1H NMR and ^{13}C NMR spectra were recorded on a 400-MHz spectrometer (400 MHz for ^1H, 100 MHz for ^{13}C). Chemical shifts are given in ppm downfield from internal tetramethylsilane for spectra recorded in $CDCl_3$. Flash column chromatography was performed employing 230–400 mesh silica gel.

Thin layer chromatography was taken using silica gel 60 F254 precoated plates (0.25 mm thickness) with a fluorescent indicator. Visualization on TLC was achieved by UV light (254 nm) and a typical TLC indication solution (cerium sulfate/molybdic acid solution). Solutions were concentrated at below 40°C under reduced pressure.

p-Methoxyphenyl (2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (8)

To a mixture of 4,6-*O*-benzylidene mannose **6** (100 mg, 0.22 mmol) and 4Å molecular sieves in CH_2Cl_2 (2 mL) were added phthalic anhydride (36 mg, 9.246 mmol) and DBU (38 μL, 0.269 mmol). After being stirred for 15 min at rt, the reaction mixture was cooled down to −78°C. DTBMP (100 mg, 0.049 mmol) and Tf_2O (57.4 μL, 0.36 mmol) were added to the reaction mixture and it was stirred for a further 15 min at −78°C. After slow addition of glycosyl acceptor **7** in CH_2Cl_2 (2 mL) at −78°C , the reaction mixture was stirred for a further 15 min, warmed up to 0°C, and further stirred for 1 h. The reaction mixture was diluted with CH_2Cl_2 and the filtered organic solution was washed with saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography (hexane/EtOAc, 2:1) to give compound **8** (167 mg, 74%): colorless oil, $R_f = 0.45$ (hexane/EtOAc, 2:1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$)δ 3.64 (d, $J = 3.2$ Hz, 1H), 3.67 (s, 3H), 3.84 (t, $J = 10.4$ Hz, 1H), 4.07 (m, 2H), 4.23 (m, 2H), 4.27 (m, 3H), 4.30 (d, $J = 3.2$ Hz, 1H), 4.45 (d, $J = 2.8$ Hz, 1H), 4.48 (dd, $J = 8.0$, 7.2 Hz, 1H), 4.58 (s, 1H), 4.60 (d, $J = 4.0$ Hz, 2H), 5.10 (d, $J = 8.0$ Hz, 1H), 5.52 (s, 1H), 5.88 (d, $J = 3.2$ Hz, 1H), 6.00 (dd, $J = 10.0$, 5.0 Hz, 1H), 6.60 (d, $J = 9.2$ Hz, 2H), 6.92 (d, $J = 9.2$ Hz, 2H), 7.09–8.20 (m, 30H). ^{13}C NMR (100 MHz, $CDCl_3$)δ 55.8, 63.3, 68.3, 68.7, 70.6, 72.0, 72.2, 72.6, 73.0, 74.9, 76.1, 77.1, 77.8, 101.1, 101.6, 103.3, 114.7, 119.0, 126.3, 127.5, 127.6, 127.7, 128.3, 128.4, 128.8, 129.1, 129.6, 129.8, 129.9, 130.0, 130.5, 133.6, 133.8, 133.9, 137.8, 138.4, 138.7, 151.5, 155.9, 165.5, 165.9, 166.4. Anal. Calcd for $C_{61}H_{56}O_{15}$: C, 71.19; H, 5.48. Found: C, 71.36; H, 5.28.

p-Methoxyphenyl (2,3-di-*O*-benzyl-β-D-mannopyranosyl)-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (9)

To a solution of disaccharide **8** (300 mg, 0.291 mmol) in CH_2Cl_2 (4 mL) were added TFA (740 μL, 4.4 mmol) and water (74 μL) and the reaction mixture was stirred for 4 h at rt. The reaction mixture was diluted with CH_2Cl_2, and washed with saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography (hexane/EtOAc, 1:2) to give dihydroxy compound

9 (183 mg, 67%): colorless oil, $R_f = 0.3$ (hexane/EtOAc, 1:2, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.00 (dd, $J = 2.8$, 3.2 Hz, 1H), 3.23 (m, 1H), 3.53 (d, $J = 2.8$ Hz, 1H), 3.69 (s, 3H), 3.79 (d, $J = 9.6$ Hz, 1H), 3.84 (d, $J = 3.2$ Hz, 1H), 3.87 (d, $J = 2.4$ Hz, 1H), 3.90 (d, $J = 12.0$ Hz, 1H), 4.10 (m, 2H), 4.13 (d, $J = 3.6$ Hz, 1H), 4.26 (t, $J = 6.4$ Hz, 2H), 4.30 (d, $J = 12.4$ Hz, 1H), 4.46 (d, $J = 12.4$ Hz, 1H), 4.50 (s, 1H), 4.55 (d, $J = 4.4$ Hz, 2H), 5.12 (d, $J = 8.4$ Hz, 1H), 5.96 (dd, $J = 10.0$, 5.0 Hz, 1H), 6.08 (d, $J = 3.6$ Hz, 1H), 6.62 (d, $J = 9.2$ Hz, 2H), 6.93 (d, $J = 9.2$ Hz, 2H), 7.00–8.19 (m, 25H). ^{13}C NMR (100 MHz, $CDCl_3$)δ 55.9, 66.1, 70.7, 70.8, 71.6, 72.2, 73.1, 74.0, 76.6, 76.8, 77.1, 79.8, 80.7, 101.2, 103.9, 114.7, 119.1, 127.5, 128.0, 128.3, 128.5, 128.8, 128.9, 129.0, 129.4, 129.9, 130.0, 130.1, 130.1, 130.6, 133.6, 133.8, 134.0, 137.8, 138.7, 151.5, 156.0, 165.5, 166.4, 166.8. Anal. Calcd for $C_{54}H_{52}O_{15}$: C, 68.93; H, 5.57. Found: C, 68.87; H, 5.51.

p-Methoxyphenyl (6-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl)-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (10)

A solution of dihydroxy disaccharide **9** (150 mg, 0.16 mmol), benzoyl chloride (33 μL, 0.191 mmol), and pyridine (150 μL) in CH_2Cl_2 (5 mL) was stirred at 0°C for 1 h. The reaction mixture was diluted with CH_2Cl_2 and washed with 1*N* HCl, saturated aqueous $NaHCO_3$ solution, and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography (hexane/EtOAc, 1:1) to afford compound **10** (144 mg, 87%): colorless oil, $R_f = 0.3$ (hexane/EtOAc, 1:1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.99 (dd, $J = 2.8$, 9.2 Hz, 1H), 3.45 (m, 1H), 3.62 (d, $J = 2.4$ Hz, 1H), 3.68 (s, 3H), 3.90 (t, $J = 9.6$ Hz, 1H), 3.96 (d, $J = 12.0$ Hz, 1H), 4.06 (m, 1H), 4.18 (d, $J = 12.0$ Hz, 1H), 4.22 (d, $J = 3.6$ Hz, 1H), 4.35 (d, $J = 12.0$ Hz, 1H), 4.47 (m, 2H), 4.57 (d, $J = 2.4$ Hz, 2H), 4.60 (m, 3H), 5.01 (d, $J = 8.0$ Hz, 1H), 5.93 (d, $J = 3.2$ Hz, 1H), 5.97 (dd, $J = 10.0$, 5.0 Hz, 1H), 6.60 (d, $J = 9.2$ Hz, 2H), 6.90 (d, $J = 9.2$ Hz, 2H), 7.00–8.10 (m, 30H). ^{13}C NMR (100 MHz, $CDCl_3$)δ 55.8, 63.3, 63.9, 66.5, 70.5, 70.9, 72.0, 72.6, 73.2, 73.9, 74.8, 80.4, 80.9, 101.2, 103.1, 114.6, 119.0, 122.7, 125.0, 126.5, 127.3, 127.9, 128.2, 128.7, 129.0, 129.5, 129.8, 129.9, 130.0, 130.3, 133.2, 133.4, 133.8, 142.4, 147.3, 149.7, 151.1, 151.4, 155.8, 165.4, 165.6, 166.2, 166.8. Anal. Calcd for $C_{61}H_{56}O_{16}$: C, 70.10; H, 5.40. Found: C, 70.07; H, 5.32.

p-Methoxyphenyl (2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→4)-(6-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl)-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (4)

To a mixture of glycosyl acceptor **10** (87 mg, 0.083 mmol) and 4Å molecular sieves in CH_2Cl_2 (4 mL) at 78°C were added sequentially DTBMP (51 mg, 0.25 mmol), Tf_2O (60 μL, 0.125 mmol), and then glycosyl donor **5** (74 mg,

0.125 mmol) in CH_2Cl_2 (4 mL). After being stirred for 30 min at −78°C, the reaction mixture was warmed up to 0°C, further stirred for 1.5 h, and diluted with CH_2Cl_2. The filtered organic solution was washed with saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography (hexane/EtOAc/CH_2Cl_2, 4:1:1) to give compound **4** (92 mg, 75%): colorless oil, $R_f = 0.25$ (hexane/EtOAc, 2:1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$)δ 2.92 (m, 1H), 3.09 (dd, $J = 2.8$, 9.2 Hz, 1H), 3.25 (dd, $J = 2.8$, 9.2 Hz, 1H), 3.44 (dd, $J = 2.8$, 9.6 Hz, 1H), 3.51 (m, 1H), 3.59 (t, $J = 3.4$ Hz, 1H), 3.69 (s, 3H), 3.82 (dd, $J = 10.4$, 4.4 Hz, 2H), 3.88 (d, $J = 2.8$ Hz, 2H), 4.04 (t, $J = 9.6$ Hz, 2H), 4.08 (m, 1H), 4.21 (m, 2H), 4.33 (m, 2H), 4.48 (d, $J = 4.4$ Hz, 1H), 4.51 (m, 2H), 4.55 (d, $J = 3.6$ Hz, 2H), 4.58 (d, $J = 1.6$ Hz, 1H), 4.64 (d, $J = 2.4$ Hz, 1H), 4.76 (d, $J = 8.0$ Hz, 1H), 4.87 (d, $J = 12.0$ Hz, 1H), 5.01 (d, $J = 8.0$ Hz, 1H), 5.46 (s, 1H), 5.93 (m, 2H), 6.60 (d, $J = 9.0$ Hz, 2H), 6.89 (d, $J = 9.0$ Hz, 2H), 7.02–8.08 (m, 45H). ^{13}C NMR (100 MHz, $CDCl_3$)δ 55.7, 61.5, 63.3, 63.4, 67.0, 67.5, 68.6, 70.3, 71.3, 71.8, 72.6, 73.8, 74.0, 74.3, 75.3, 75.4, 77.0, 78.5, 78.6, 79.7, 101.1, 101.5, 101.8, 102.7, 114.5, 119.0, 126.3, 127.2, 127.7, 127.7, 127.8, 127.9, 128.3, 128.4, 128.5, 128.6, 129.0, 129.3, 129.7, 129.9, 130.0, 133.2, 133.4, 133.8, 137.8, 138.3, 138.5, 138.7, 138.8, 151.4, 155.8, 165.4, 165.5, 166.2, 166.4. Anal. Calcd for $C_{88}H_{82}O_{21}$: C, 71.63; H, 5.60. Found: C, 71.67; H, 5.47.

(2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→4)-(6-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl)-(1→3)-2,4,6-tri-*O*-benzoyl-D-galactopyranose (2)

To a mixture of compound **4** (59 mg, 0.04 mmol) in a mixed solvent of CH_3CN (800 μL) and H_2O (200 μL) at 0°C was added CAN (44 mg, 0.08 mmol). After being stirred for 30 min at 0°C, the reaction mixture was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography (hexane/EtOAc/CH_2Cl_2, 2:1:1) to afford compound **2** (33 mg, 60%): yellow oil, $R_f = 0.18$ (hexane/EtOAc, 2:1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$)δ 2.97 (m, 1H), 3.30 (dd, $J = 2.8$, 9.2 Hz, 1H), 3.32 (s, 1H), 3.40 (d, $J = 1.6$ Hz, 1H), 3.46 (dd, $J = 2.8$, 9.2 Hz, 1H), 3.61 (m, 2H), 3.86 (dd, $J = 4.4$, 4.8 Hz, 1H), 3.90 (m, 1H), 4.06 (dd, $J = 9.6$, 8.0 Hz, 2H), 4.16 (d, $J = 12.0$ Hz, 1H), 4.21 (d, $J = 12.0$ Hz, 1H), 4.28 (d, $J = 6.4$ Hz, 1H), 4.34 (dd, $J = 5.2$, 5.6 Hz, 2H), 4.48 (m, 3H), 4.47 (d, $J = 12.0$ Hz, 2H), 4.55 (m, 3H), 4.60 (d, $J = 5.2$ Hz, 1H), 4.67 (d, $J = 7.2$ Hz, 1H), 4.82 (m, 1H), 4.90 (d, $J = 12.0$ Hz, 1H), 5.47 (s, 1H), 5.69 (d, $J = 9.2$ Hz, 1H), 5.96 (s, 1H), 7.05–8.08 (m, 45H). ^{13}C NMR (100 MHz, $CDCl_3$)δ 63.3, 63.5, 67.5, 67.8, 68.6, 71.3, 71.4, 71.7, 72.4, 72.6, 73.8, 73.8, 73.9, 74.2, 75.4, 77.4, 78.5, 78.6, 79.7, 91.2, 101.5, 102.1, 102.9, 126.3, 127.0, 127.2, 127.6, 127.7, 127.9, 128.0, 128.3, 128.4, 128.5, 128.6, 128.6, 129.0, 129.0, 129.1, 129.6, 129.7, 129.9, 129.9, 130.0,

130.1, 130.2, 133.1, 133.2, 133.3, 133.8, 138.2, 165.4, 165.9, 166.3, 166.4. Anal. Calcd for $C_{81}H_{76}O_{20}$: C, 71.04; H, 5.59. Found: C, 71.02; H, 5.58.

p-Methoxyphenyl (2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-(6-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl)-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (3)

To a solution of compound **4** (67 mg, 0.0454 mmol) in THF (1mL) at 0°C were added $BH_3 \cdot$ THF (450 μL) and Bu_2BOTf (45 μL). After being stirred for 15 min at 0°C, the reaction mixture was warmed up to rt, stirred for a further 2 h, and neutralized with Et_3N. MeOH was added to the resulting mixture, which was then concentrated in vacuo. The residue was purified by flash column chromatography (hexane/EtOAc/CH_2Cl_2, 2:1:1) to afford compound **3** (50 mg, 75%): colorless oil, $R_f = 0.125$ (hexane/EtOAc, 2:1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.97 (m, 1H), 3.19 (dd, $J = 2.8$, 9.2 Hz, 1H), 3.35 (dd, $J = 2.8$, 9.2 Hz, 1H), 3.43 (m, 2H), 3.58 (m, 2H), 3.63 (d, $J = 2.8$ Hz, 1H), 3.69 (s, 3H), 3.78 (t, $J = 9.6$ Hz, 1H), 3.85 (d, $J = 2.8$ Hz, 1H), 4.02 (dd, $J = 4.4$, 4.0 Hz, 1H), 4.08 (t, $J = 9.6$ Hz, 1H), 4.16 (dd, $J = 2.0$, 3.2 Hz, 2H), 4.20 (d, $J = 6.4$ Hz, 1H), 4.28 (d, $J = 12.0$ Hz, 1H), 4.38 (d, $J = 3.2$ Hz, 2H), 4.42 (m, 2H), 4.46 (m, 1H), 4.49 (d, $J = 3.2$ Hz, 1H), 4.52 (d, $J = 3.6$ Hz, 1H), 4.55 (d, $J = 3.2$ Hz, 2H), 4.57 (d, $J = 3.6$ Hz, 1H), 4.60 (d, $J = 3.6$ Hz, 1H), 4.77 (d, $J = 12.0$ Hz, 1H), 4.82 (d, $J = 4.4$ Hz, 1H), 5.00 (d, $J = 8$ Hz, 1H), 5.90 (d, $J = 3.2$ Hz, 1H), 5.95 (dd, $J =$ 8.0, 5.0 Hz, 1H), 6.60 (d, $J = 9.2$ Hz, 2H), 6.90 (d, $J = 9.2$ Hz, 2H), 7.01–8.09 (m, 45H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.8, 62.4, 63.3, 63.8, 70.4, 70.9, 71.5, 71.8, 72.5, 73.8, 74.1, 74.6, 74.7, 74.9, 75.3, 75.8, 76.9, 78.1, 78.4, 82.6, 100.3, 101.1, 102.8, 114.6, 119.0, 127.3, 127.4, 127.6, 127.7, 127.8, 127.9, 128.0, 128.2, 128.2, 128.2, 128.3, 128.4, 128.6, 128.6, 128.6, 128.7, 128.8, 128.9, 129.4, 129.8, 130.0, 130.0, 130.1, 130.2, 130.3, 130.4, 133.3, 133.4, 133.8, 138.1, 138.3, 151.4, 155.8, 165.4, 165.5, 166.2, 166.5. Anal. Calcd for $C_{88}H_{84}O_{21}$: C, 71.53; H, 5.73. Found: C, 71.49; H, 5.85.

p-Methoxyphenyl (2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→4)-(6-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-(6-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl)-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (1)

To a mixture of trisaccharide donor **2** (100 mg, 0.072 mmol) and 4Å molecular sieves in CH_2Cl_2 (2 mL) were added phthalic anhydride (12 mg, 0793 mmol) and DBU (13.3 μL, 0.086 mmol). After being stirred for 15 min at rt,

the reaction mixture was cooled down to −78°C. DTBMP (32.4 mg, 0.158 mmol) and Tf_2O (52 μL, 0.108 mmol) were added to the reaction mixture and it was stirred for 15 min at −78°C. After slow addition of glycosyl acceptor **3** (127 mg, 0.086 mmol) in CH_2Cl_2 (2 mL), the reaction mixture was stirred for 15 min, warmed up to 0°C, further stirred for 1.5 h, and diluted with CH_2Cl_2. The filtered organic solution was washed with saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography (hexane/EtOAc, 2:1) and high-pressure gel permeation chromatography to give hexasaccharide **1** (140 mg, 69%): colorless oil, $R_f = 0.24$ (hexane/EtOAc, 2:1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.90 (m, 1H), 3.10 (d, $J = 10.4$ Hz, 1H), 3.24 (dd, $J = 2.4$, 8.8 Hz, 1H), 3.41 (dd, $J = 2.8$, 6.4 Hz, 2H), 3.49 (d, $J = 2.8$ Hz, 1H), 3.54 (m, 2H), 3.67 (s, 3H), 3.68 (m, 1H), 3,78 (m, 1H), 3.80 (d, $J = 2.4$ Hz, 1H), 3.98–4.15 (m, 12H), 4.21–4.45 (m, 14H), 4.47–4.59 (m, 14H), 4.63 (d, $J = 4.8$ Hz, 1H), 4.66 (m, 1H), 4.75 (m, 1H), 4.79 (m, 1H), 4.85 (m, 1H), 5.00 (d, $J = 8$ Hz, 1H), 5.45 (s, 1H), 5.71 (t, $J = 9.2$ Hz, 1H), 5.80 (d, $J = 2.8$ Hz, 1H), 5.92 (d, $J = 4.0$ Hz, 1H), 6.60–8.06 (m, 94H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.8, 60.6, 62.9, 63.3, 63.6, 64.2, 67.5, 68.4, 68.6, 70.4, 70.7, 70.8, 71.2, 71.5, 71.7, 71.9, 72.5, 72.6, 73.2, 73.6, 73.8, 73.8, 74.1, 74.2, 74.5, 75.1, 75.3, 75.4, 77.0, 77.3, 77.4, 77.5, 77.6, 78.0, 78.2, 78.6, 78.7, 79.8, 80.0, 82.7, 100.7, 101.1, 101.5, 101.9, 102.7, 102.8, 103.0, 114.5, 119.0, 126.3, 126.8, 127.1, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.3, 128.3, 128.3, 128.4, 128.4, 128.5, 128.5, 128.6, 128.6, 128.6, 128.9, 129.0, 129.3, 129.8, 129.9, 129.9, 130.0, 130.2, 133.1, 133.2, 133.4, 133.8, 138.8, 138.9, 151.4, 155.8, 165.3, 165.4, 165.5, 165.5, 166.2, 166.2, 166.3, 166.4. Anal. Calcd for $C_{169}H_{158}O_{40}$: C, 71.75; H, 5.63. Found: C, 71.75; H, 5.60.

REFERENCES

1. Pagani, H.; Parenti, F. *Kineosporia*, a new genus of the order *Actinomycetales*. *Int. J. Syst. Bacteriol.* **1978,** *28*, 401–406.

2. Itoh, T.; Kudo, T.; Parenti, F.; Seino, A. Amended description of the genus *Kineosporia*, based on chemotaxonomic and morphological studies. *Int. J. Syst. Bacteriol.* **1989,** *39*, 168–173.

3. Kudo, T.; Matsushima, K.; Itoh, T.; Sasaki, J.; Suzuki, K.-I. Description of four new species of the genus *Kineosporia*: *Kinosporia succinea* sp. nov., *Kinosporia rhizophila* sp. nov., *Kinosporia mikuniensis* sp. nov. and *Kineosporia rhamnosa* sp. nov., isolated form plant samples, and amended description of the genus *Kineosporia*. *Int. J. Syst. Bacteriol.* **1998,** *48*, 1245–1255.

4. Naumova, I.B.; Shashkov, A.S.; Tul'skaya, E.M.; Streshinskaya, G.M.; Kozlova, Y.I.; Potekhina, N.V.; Evtushenko, L.I.; Stackebrandt, E. Cell wall teichoic acids: structural diversity, species specificity in the genus *Nocardiopsis*, and chemotaxonomic perspective. *FEMS Microbiol. Rev.* **2001,** *25*, 269–284.

5. Tul'skaya, E.M.; Senchenkova, S.N.; Evtushenko, L.I.; Shashkov, A.S.; Naumova, I.B. A new neutral polymer from the cell wall of actinomycete *Kineosporia aurantiaca* VKM Ac-702^T. *Carbohydr. Res.* **2005,** *340*, 1247–1251.

6. For reviews on the β-mannopyranosylation, see: (a) Barresi, F.; Hindsgaul, O. Synthesis of β-D-mannose containing oligosaccharides. In *Modern Methods in Carbohydrate Synthesis*; Khan, S.H., O'Neil, R.A., Eds.; Harwood Academic Publishers: Amsterdam, 1996, 251–276. (b) Gridley, J.J.; Osborn, H.M.I. Recent advances in the construction of β-D-mannose and β-D-mannosamine linkages. *J. Chem. Soc. Perkin Trans. 1* **2000,** 1471–1491. (c) Demchenko, A.V. 1,2-*cis O*-Glycosylation: methods, strategies, principles. *Curr. Org. Chem.* **2003,** *7*, 35–79.

7. (a) Crich, D.; Sun, S. Formation of β-mannopyranosides of primary alcohols using the sulfoxide method. *J. Org. Chem.* **1996,** *61*, 4506–4507. (b) Crich, D.; Sun, S. Direct synthesis of β-mannopyranosides by the sulfoxide method. *J. Org. Chem.* **1997,** *62*, 1198–1199. (c) Crich, D.; Sun, S. Direct chemical synthesis of β-mannopyranosides and other glycosides via glycosyl triflates. *Tetrahedron* **1998,** *54*, 8321–8348. (d) Crich, D.; Sun, S. Direct formation of β-mannopyranosides and other hindered glycosides from thioglycosides. *J. Am. Chem. Soc.* **1998,** *120*, 435–436.

8. Kim, K.S.; Fulse, D.B.; Baek, J.Y.; Lee, B.-Y.; Jeon, H.B. Stereoselective direct glycosylation with anomeric hydroxy sugars by activation with phthalic anhydride and trifluoromethanesulfonic anhydride involving glycosyl phthalate intermediates. *J. Am. Chem. Soc.* **2008,** *130*, 8537–8547.

9. Kim, K.S.; Kim, J.H.; Lee, Y.J.; Park, J. 2-(Hydroxycarbonyl)benzyl glycosides: a novel type of glycosyl donors for highly efficient β-mannopyranosylation and oligosaccharide synthesis by latent-active glycosylation. *J. Am. Chem. Soc.* **2001,** *123*, 8477–8481.

10. (a) Kwon, Y.T.; Lee, Y.J.; Lee, K.; Kim, K.S. Synthesis of the trisaccharide repeating unit of the atypical O-antigen polysaccharide from Danish Helicobacter pylori strains employing the 2′-carboxybenzyl glycoside. *Org. Lett.* **2004,** *6*, 3901–3904. (b) Lee, B.R.; Jeon, J.M.; Jung, J.H.; Jeon, H.B.; Kim, K.S. Synthesis of the tetrasaccharide repeat unit of the O-antigen polysaccharide from *Escherichia coli* O77 employing the 2′-carboxybenzyl glycoside. *Can. J. Chem.* **2006,** *84*, 506–515. (c) Lee, Y.J.; Lee, K.; Jung, E.H.; Jeon, H.B.; Kim, K.S. Acceptor-dependent stereoselective glycosylation: 2′-CB glycoside-mediated direct β-D-arabinofuranosylation and efficient synthesis of the octaarabinofuranoside in mycobacterial cell wall. *Org. Lett.* **2005,** *7*, 3263–3266. (d) Lee, B.-Y.; Baek, J.Y.; Jeon, H.B.; Kim, K.S. Improved synthesis of the tetrasaccharide repeat unit of the O-antigen polysaccharide from *Escherichia coli* O77. *Bull. Korean Chem. Soc.* **2007,** *28*, 257–262. (e) Lee, Y.J.; Fulse, D.B.; Kim, K.S. Synthesis of a tetrasaccharide phosphate from the linkage region of the arabinogalactan-peptidoglycan complex in the mycobacterial cell wall. *Carbohydr. Res.* **2008,** *343*, 1574–1584. (f) Baek, J.Y.; Joo, Y.J.; Kim, K.S. Stereoselective α -galactofuranosylation and synthesis of di- and tetrasaccharide subunits of cell wall polysaccharides of *Talaromyces flavus*. *Tetrahedron Lett.* **2008,** *49*, 4734–4737.

11. Codee, J.D.C.; Hossain, L.H.; Seeberger, P.H. Efficient installation of β-mannosides using a dehydrative coupling strategy. *Org. Lett.* **2005,** *7*, 3251–3254.

12. (a) Bazin, H.G.; Du, Y.; Polat, T.; Linhardt, R.J. Synthesis of a versatile neuraminic acid "*C*"-disaccharide precursor for the synthesis of *C*-glycoside analogues of Gangliosides. *J. Org. Chem.* **1999,** *64*, 7254–7259. (b) Chen, L.; Kong, F. A practical synthesis of β-D-GlcA-(1→3)-β-D-Gal-(1→3)-β-D-Gal-(1→4)-D-Xyl, a part of the common linkage region of a glycosaminoglycan. *Carbohydr. Res.* **2002,** *337*, 1373–1380.

13. Bock, K.; Pedersen, C. A study of ^{13}CH coupling constants in hexopyranoses. *J. Chem. Soc. Perkin Trans. 2* **1974,** 293–297.

14. Weingart, R.; Schmidt, R.R. Can preferential β-mannopyranoside formation with 4,6-*O*-benzylidene protected mannopyranosyl sulfoxides be reached with trichloroacetimidates? *Tetrahedron Lett.* **2000,** *41*, 8753–8758.

15. (a) Crich, D.; Li, W.; Li, H. Direct chemical synthesis of the β-Mannans: Linear and block syntheses of the alternating β-(1→3)-β-(1→4)-Mannan common to Rhodotorula glutinis, Rhodotorula mucilaginosa, and Leptospira biflexa. *J. Am. Chem. Soc.* **2004**, *126*, 15081–15086. (b) Jiang, L.; Chan, T.-H. Borane/Bu_2BOTf: a mild reagent for the regioselective reductive ring opening of benzylidene acetals in carbohydrates. *Tetrahedron Lett.* **1998,** *39*, 355–358.

16. (a) Fukiyama, T.; Laird, A.A.; Hotchkiss, L.M. *p*-Anisyl group: a versatile protecting group for primary alcohols. *Tetrahedron Lett.* **1985,** *26*, 6291–6292. (b) Mori, M.; Ito, Y.; Ogawa, T. A highly stereoselective and practical synthesis of cyclomannohexaose, cyclo→4)-[α-D-Manp-(l→4)-]$_5$-α-D-Manp-(l→, a *manno* isomer of cyclomaltohexaose. *Carbohydr. Res.* **1989,** *192*, 131–146.