Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Research 341 (2006) 1796–1802
Carbohydrate RESEARCH

Synthesis of the sugar moiety of TIME-EA4, a glycopeptide isolated from silkworm diapause eggs

Shouji Hiro, Yoshinosuke Usuki and Hideo Iio*

Department of Material Science, Graduate School of Science, Osaka City University, Osaka 558-8585, Japan

Received 21 February 2006; received in revised form 26 March 2006; accepted 12 April 2006
Available online 15 May 2006

Abstract—We describe the efficient synthesis of the tetrasaccharide, 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,4-di-*O*-acetyl-3-*O*-allyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl azide, which is the protected form of the sugar unit of TIME-EA4 that is isolated from the diapausing eggs of the silkworm, *Bombyx mori*. The β-linked D-mannoside of the tetrasaccharide was obtained using the conventional oxidation–reduction method for inversion of the configuration at the C-2 hydroxyl group of β-D-glucoside. The reduction was effected with $NaBH_4$ in a methanolic solution in a ratio of 98:2 in favor of the β-D-mannoside that was obtained in 87% yield.

Keywords: TIME-EA4; *N*-Glycan; Glycosylation; β-D-Mannoside; Oxidation–reduction

1. Introduction

Glycoproteins play an important role in biological processes such as cellular and immunogenic recognition. Moreover, the oligosaccharide moiety is thought to contribute to the solubility and thermal stability of proteins and to structural roles such as cell adhesion.[1] The sugar moieties are linked to the protein component through either *O*-glycosidic or *N*-glycosylic bonds. All *N*-glycans have a core pentasaccharide that links to the amide nitrogen in the side chain of Asn in the consensus sequence Asn-Xaa-Ser/Thr, where Xaa can be any amino acid except Pro and Asp.[2]

During the course of our studies on the synthesis of *N*-glycopeptides, we began the initial studies on the sugar moiety of TIME-EA4 (EA4), an ATPase isolated from the diapausing eggs of the silkworm, *Bombyx mori*, by Isobe and co-workers.[3] EA4 is a glycoprotein containing 156 amino acids and an N-linked tetrasaccharide, which appears to be involved in measuring the duration of diapause in the life cycle of the silkworm.[4] The glycan structure and the glycosylation site of EA4 were unambiguously determined to be Man-Man-GlcNAc-GlcNAc-Asn22 in order from the nonreducing end (Fig. 1); this was done by using an electrospray-ionization (ESI)-tandem quadrupole/orthogonal-acceleration time-of-flight (Q-TOF) mass spectrometer with a nano-HPLC system (LC–ESI-Q-TOF-MS).[5] The same sugar structure was also reported for the membrane glycoproteins of three insect cell lines.[6] Recently, a core pentasaccharide was additionally observed at the same linkage site of the peptide from the Showa strain of the silkworm.[7] In this paper, we describe an efficient synthesis of the sugar moiety of TIME-EA4 in the fully protected form.

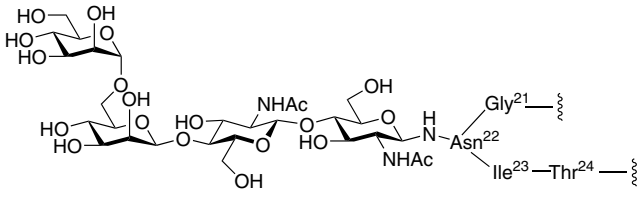


Figure 1. Proposed tetrasaccharide structure for the carbohydrate moiety attached to Asn22 in TIME-EA4.

* Corresponding author. Fax: +81 6 6605 3152; e-mail: iio@sci.osaka-cu.ac.jp

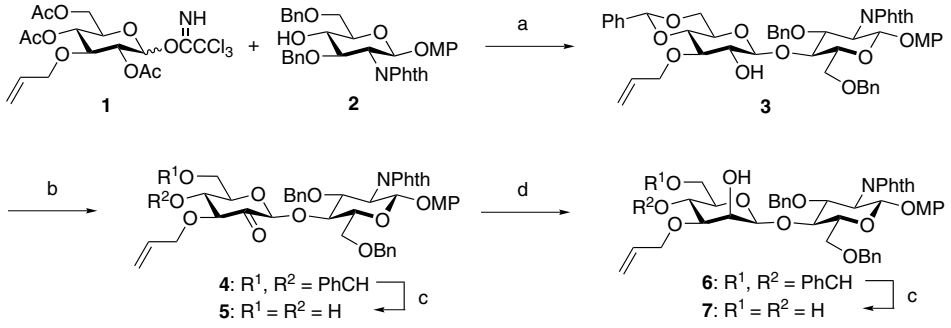
0008-6215/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2006.04.015

2. Results and discussion

The key challenges for the synthesis of *N*-glycans have been the construction of β-D-mannosides, which are especially difficult to accomplish since both neighboring group assistance and the anomeric effect uniformly favor the formation of α-D-mannosides during glycosylation. Methods for the construction of β-D-mannosidic linkages have been reviewed,[8] and several approaches, including the intramolecular aglycon delivery[9] and direct β-D-mannoside coupling protocol,[10] have been reported to effectively solve this problem. Epimerizations of β-D-glucopyranosides at the C-2 position by direct S_N2 inversion[11] or oxidation–reduction of the C-2 hydroxyl group[12] are established methods for the indirect preparation of β-D-mannosides; additionally, a two-step protocol for β-selective glycosylation using a glyco-ulosyl bromide and an acceptor, followed by manno-selective reduction of the resulting β-2-ketoglucoside, is also established.[13] In this study, we have accomplished the synthesis of the oligosaccharide by applying the conventional oxidation–reduction method of inversion of the configuration of the β-D-glucoside to yield the β-D-mannoside. In other words, our strategy for the synthesis of the β-D-mannoside was to first construct the β-(1→4)-linked D-glucosyl derivative and then invert the configuration of the C-2 hydroxyl group of the D-Glc residue according to the oxidation–reduction protocol[12] to obtain a β-(1→4)-linked D-mannosyl derivative (Scheme 1).

For this purpose, we selected the 3-*O*-allyl-protected glucosyl trichloroacetimidate **1**[14] as a donor, which was coupled with glucosamine acceptor **2**[15] using trimethylsilyl triflate (TMSOTf)[16] as a promoter to give the β-D-gluco-configured disaccharide. Prior to the conversion of the β-D-glucoside to the β-D-mannoside, the 2^{II}-OH group had to be selectively deprotected. Thus, the removal of the acetates and the regioselective benzylidenation of the intermediate yielded disaccharide **3**, which was suitably functionalized for the ensuing inversion sequence to the β-D-mannoside. First, compound **3** was oxidized by dimethyl sulfoxide (DMSO)–acetic anhydride[12] to afford uloside **4**, and the benzylidene group of the resulting compound was removed to yield diol uloside **5**.

The reductions of **4** and **5** were evaluated for the inversion sequence under several conditions. The results obtained with a series of different reagents and solvents are shown in Table 1. The reduction of the 2-keto group of ulosides **4** and **5** by sodium borohydride in a polar protic solvent yielded the corresponding β-D-mannoside as the major product with a distinctly higher stereoselectivity. In entry 1, in particular, the conventional sodium borohydride reduction of **4** in methanol stereoselectively



Scheme 1. Reagents and conditions: (a) (i) TMSOTf, CH_2Cl_2, 0 °C, 5 min; (ii) NaOMe, 3:1 MeOH–CH_2Cl_2, rt, 18 h; (iii) benzaldehyde dimethylacetal, CSA, MeCN, 56% (three steps from **1** and **2**); (b) Ac_2O, DMSO, rt, 3 h, 79%; (c) ethylene glycol, TsOH·H_2O, MeCN, rt; (d) $NaBH_4$, MeOH.

Table 1. Stereoselectivities of the hydride reductions of **4** and **5**

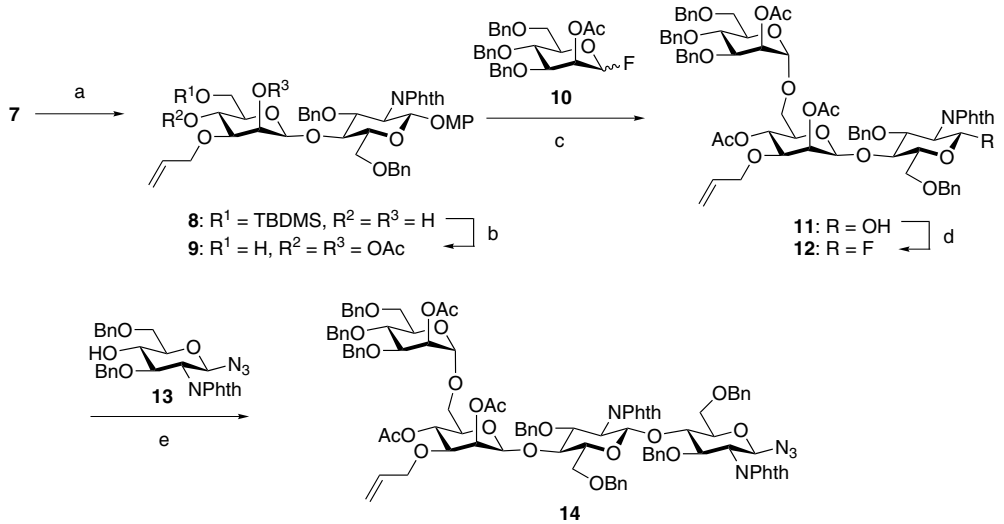
Entry	Uloside	Reagent	Solvent	Time (min)	Ratio[a] manno:gluco	Total yield (%)
1	**4**	$NaBH_4$	MeOH	1	98:2	89
2	**4**	$NaBH_4$	1:1 MeOH–CH_2Cl_2	5	87:13	84
3	**4**	$NaBH_4$	CH_2Cl_2	10	55:45	82
4	**4**	Bu_4NBH_4	THF	30	52:48	70
5	**5**	$NaBH_4$	MeOH	1	92:8	82
6	**5**	$NaBH_4$	1:1 MeOH–CH_2Cl_2	5	89:11	76
7	**5**	$NaBH_4$	CH_2Cl_2	10	10:90	88
8	**5**	Bu_4NBH_4	THF	30	5:95	75

[a] The mixture of manno- and gluco-isomers was separated by column chromatography.

provided β-D-mannoside **6**, an epimer of **3**, in excellent yield. On the other hand, sodium borohydride reduction in 1:1 dichloromethane–methanol, the solvent system recommended for manno-selective reduction,[13] resulted in unsatisfactory stereoselectivity. The epimeric β-D-glucoside and β-D-mannoside were readily separated by column chromatography to afford pure compounds. The stereochemistry of the reduction products was confirmed by ^{1}H NMR analyses of **6** and **7**. Curiously, reduction of the deprotected compound **5** by sodium borohydride or tetrabutylammonium borohydride in an aprotic solvent provided stereoselectivity in favor of the β-D-glucoside (entries 7 and 8), while the benzylidene compound **4** was reduced nonselectively under identical conditions (entries 3 and 4). A reversal in the carbonyl reduction selectivities of ulosides was also reported: 3,4,6-*O*-tribenzoylulosides are reduced by the borane·pyridine complex in tetrahydrofuran (THF) in a gluco:manno ratio greater than 8:1.[13b] The reasons for manno:gluco selectivity are not clearly apparent at the moment.

In order to obtain the tetrasaccharide, a mannoside unprotected at the 6-O position was required. Therefore, as depicted in Scheme 2, compound **6** was treated with ethylene glycol and *p*-toluenesulfonic acid in acetonitrile for 10 min to obtain triol **7** in 92% yield. Subsequently, **7** was selectively protected at the 6-position with a bulky *tert*-butyldimethylsilyl group[17] to provide **8** in 78% yield. Diol **8** was acetylated with acetic anhydride and triethylamine; this was followed by deprotection of the silyl group with hydrogen fluoride[18] to give **9** in 92% yield in two steps. Next, having obtained **9**, we proceeded to the synthesis of the protected tetrasaccharide by using the fluoride donor **10**[19] and the azide acceptor **13**.[19] The reaction between **9** and the fluoride donor **10** in the presence of boron trifluoride etherate ($BF_3{\cdot}OEt_2$) proceeded smoothly to yield the trisaccharide as a single α-anomer, the *p*-methoxyphenyl (MP) group of which was oxidatively cleaved by ceric ammonium nitrate (CAN)[20] to provide **11** in 68% yield in two steps. Subsequently, treatment with dimethylaminosulfur trifluoride (DAST)[21] for 10 min at 0 °C afforded fluoride **12**, which was finally glycosylated with the azide acceptor **13** in the presence of $BF_3{\cdot}OEt_2$ to yield the desired tetrasaccharide **14** as a single β-anomer. The stereostructures of **12** and **14** were fully characterized by 1D and 2D ^{1}H NMR spectroscopy.

In summary, we achieved a stereocontrolled synthesis of the protected tetrasaccharide **14** in TIME-EA4, a *N*-glycopeptide isolated from diapausing eggs of the silkworm, *B. mori*. The β-linked D-mannoside of the tetrasaccharide was formed using an oxidation–reduction inversion sequence on the β-D-glucoside. The reduction was effected with $NaBH_4$ in a methanolic solution in a ratio of 98:2 in favor of the β-D-mannoside that was obtained in 87% yield. Compound **14** is fully functionalized and protected for further manipulation toward the synthesis of the *N*-glycopeptide. The glycosylasparagine of EA4 could be synthesized by the reaction between tetrasaccharide **14** and Fmoc-aspartic acid *tert*-butyl ester in the presence of triethylphosphine,[22] which would be used as the oligosaccharide donor to *N*-acetylglucosaminyl peptide using the transglycosylation activity of *endo*-β-*N*-acetylglucosaminidase.[23] This oxidation–reduction strategy for the synthesis of the sugar moiety of the *N*-glycan could be applicable to the other higher sugar derivatives and is under investigation.



Scheme 2. Reagents and conditions: (a) TBDMSCl, NEt_3, DMF, rt, 78%; (b) (i) Ac_2O, Et_3N, DMAP, rt; (ii) HF·py, CH_2Cl_2, 0 °C, 91% (two steps from **8**); (c) (i) **10**/$BF_3{\cdot}OEt_2$, CH_2Cl_2, 10 min, 0 °C; (ii) CAN, 10:1 MeCN–H_2O, 5 min, 68% (two steps from **9** and **10**); (d) DAST, CH_2Cl_2, 0 °C, 78%; (e) **13**/$BF_3{\cdot}OEt_2$, CH_2Cl_2, 10 min, 0 °C, 70%.

3. Experimental

3.1. General methods

^{1}H and ^{13}C NMR spectra were recorded, respectively, at 600 and 150 MHz (Bruker DRX-600), 400 and 100 MHz (JEOL JNMLA400) or 300 and 75 MHz (JEOL JNMLA300). Chemical shifts are reported in ppm relative to Me_4Si and $CDCl_3$ with $CHCl_3$ as the internal reference (7.26 ppm for ^{1}H NMR and 77.0 ppm for ^{13}C NMR). Coupling constants are reported in hertz (Hz) and determined directly from ^{1}H NMR spectra. Spectral splitting patterns were designated as s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad). Mass spectra were obtained on JEOL JMS-700T or JEOL JMS-AX500 spectrometers.

All air- and moisture-sensitive reactions were carried out in flame-dried, argon-flushed, two-necked flasks sealed with rubber septa, and dry solvents and reagents were introduced with a syringe. THF and Et_2O were freshly distilled from sodium benzophenone ketyl. CH_2Cl_2 was freshly distilled from P_2O_5. Flash column chromatography was carried out on KANTO CHEMICAL silica gel 60 N (spherical, neutral, 40–50 μm), and precoated E. Merck silica gel plates (Art5715 Kieselgel $60F_{257}$ 0.25 mm) were used for TLC analyses.

3.2. *p*-Methoxyphenyl 3-*O*-allyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (3)

A mixture of acceptor **2** (0.12 g, 0.20 mmol), donor **1** (0.15 g, 0.31 mmol) and 4 Å molecular sieves (MS, 0.10 g) in CH_2Cl_2 (2.0 mL) was stirred for 1 h. TMSOTf (10 μL, 52 μmol) was added at 0 °C. After stirring for 5 min, the reaction was quenched with aq $NaHCO_3$. The mixture was extracted with CH_2Cl_2, and the organic layer was washed with brine and dried (Na_2SO_4). Evaporation of solvent gave the crude β-D-gluco-configured disaccharide, which was dissolved in 3:1 MeOH–CH_2Cl_2 (3.0 mL) and treated with NaOMe (11 mg) overnight at room temperature. After neutralization with an excess of Amberlite IR-120 (H^+) cation-exchange resin, filtration and evaporation of the solvent gave the crude tetraol. This was taken up in MeCN (1.0 mL) and treated with benzaldehyde dimethylacetal (45 μL, 0.30 mmol) and D-camphor-10-sulfonic acid monohydrate (23 mg, 0.10 mmol) for 1 h. The reaction was quenched by the addition of Et_3N, and the solvent was evaporated to dryness. Flash chromatography of the residue gave **3** (0.10 g, 0.11 mmol, 55%, three steps from **2** and **8**): $[\alpha]_D^{20}$ +38 (*c* 0.06, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.67–6.66 (23H, m, arom), 6.02–5.89 (1H, m, CH_2CH–CH_2–), 5.60 (1H, d, *J* 8.4 Hz, H-1^I), 5.46 (1H, s, benzylidene), 5.31 (1H, dd, *J* 1.5 and 17.1 Hz, CH_2CH–CH_2–), 5.20 (1H, dd, *J* 1.5 and 11.6 Hz, CH_2CH–CH_2–), 4.81, 4.60 (1H, d, *J* 12.3 Hz, OCH_2Ph), 4.79 (1H, d, *J* 11.9 Hz, OCH_2Ph), 4.68 (1H, d, *J* 7.3 Hz, H-1^{II}), 4.46–4.38 (4H, m, H-2^I, OCH_2Ph, CH_2CH–CH_2–), 4.24–4.15 (3H, m, H-3^I, 4^{I,II}), 4.05 (1H, dd, *J* 3.7 and 11.0 Hz, H-6a^I), 3.85 (1H, dd, *J* 1.8 and 11.0 Hz, H-6b^I), 3.70 (3H, s, OMe), 3.75–3.66 (1H, m, H-5^I), 3.56–3.42 (4H, m, H-2^{II}, 3^{II}, 6^{II}), 3.27–3.19 (1H, m, H-5^{II}), 3.06 (1H, s, OH). ^{13}C NMR ($CDCl_3$, 100 MHz, selected signals): δ 101.13 (C-1^{II}), 97.73 (C-1^I). HRFABMS (positive-ion mode): Calcd for $C_{51}H_{52}NO_{13}$ $[M+H]^+$, 886.3440; found, 886.3416.

3.3. *p*-Methoxyphenyl 3-*O*-allyl-4,6-*O*-benzylidene-β-D-*arabino*-hex-2-ulopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (4)

A solution of **3** (85 mg, 96 μmol) in 1:2 Ac_2O–DMSO (3.0 mL) was stirred for 3 h at room temperature. The reaction was quenched with aq $NaHCO_3$. The mixture was extracted with Et_2O and the organic layer was washed with brine, dried (Na_2SO_4), and concentrated. Flash chromatography of the residue gave **4** (67 mg, 76 μmol, 79%): $[\alpha]_D^{20}$ +10 (*c* 0.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.68–6.68 (23H, m, arom), 6.02–5.86 (1H, m, CH_2CH–CH_2–), 5.59 (1H, d, *J* 10.8 Hz, H-1^I), 5.45 (1H, s, benzylidene), 5.31 (1H, dd, *J* 1.5 and 17.2 Hz, CH_2CH–CH_2–), 5.21 (1H, dd, *J* 1.5 and 10.2 Hz, CH_2CH–CH_2–), 4.86, 4.49 (1H, d, *J* 10.0 Hz, OCH_2Ph), 4.77, 4.65 (1H, d, *J* 11.9 Hz, OCH_2Ph), 4.59 (1H, s, H-1^{II}), 4.46–4.34 (4H, m, H-2^I, 3^I, CH_2CH–CH_2–), 3.84–3.57 (6H, m, H-4^{II}, 5^I, 6^{I,II}), 3.71 (3H, s, OMe), 3.53 (1H, d, *J* 10.7 Hz, H-3^{II}), 3.25–3.18 (1H, m, H-5^{II}), small peaks were additionally observed due to the ketone hydrate of compound **4**.[12c] HRFABMS (positive-ion mode): Calcd for $C_{51}H_{50}NO_{13}$ $[M+H]^+$, 884.3212; found, 884.3274.

3.4. *p*-Methoxyphenyl 3-*O*-allyl-β-D-*arabino*-hex-2-ulopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (5)

To a solution of **4** (67 mg, 76 μmol) and ethylene glycol (50 μL) in MeCN (1.0 mL) was added *p*-TsOH·H_2O (13 mg, 68 μmol). After stirring for 30 min at room temperature, the reaction was quenched with aq $NaHCO_3$. The mixture was diluted with Et_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and concentrated. Flash chromatography of the residue gave **5** (46 mg, 57 μmol, 75%): $[\alpha]_D^{20}$ +40 (*c* 0.19, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.68–6.67 (18H, m, arom), 6.02–5.89 (1H, m, CH_2CH–CH_2–), 5.59 (1H, d, *J* 8.4 Hz, H-1^I), 5.34 (1H, dd, *J* 1.5 and 17.3 Hz, CH_2CH–CH_2–), 5.21 (1H, dd, *J* 1.5 and 10.0 Hz, CH_2CH–CH_2–), 5.12 (1H, s, H-1^{II}), 4.91, 4.48 (1H, d, *J* 11.9 Hz, OCH_2Ph), 4.70, 4.64 (1H, d, *J* 11.9 Hz, OCH_2Ph), 4.59 (1H, dd, *J* 8.3 and 10.6 Hz, H-3^I), 4.46

(1H, dd, *J* 8.4 and 10.6 Hz, H-2^{I}), 4.35–4.23 (2H, m, CH_2CH–CH_2–), 4.13 (1H, dd, *J* 8.3 and 9.6 Hz, H-4^{I}), 4.13–4.08 (1H, m, H-5^{II}), 3.92–3.74 (6H, m, H-4^{II}, 5^{I}, 6^{I,II}), 3.71 (3H, s, OMe), 2.06 (1H, d, *J* 9.7 Hz, H-3^{II}). HRFABMS (negative-ion mode): Calcd for $C_{44}H_{45}NO_{13}$ $[M]^-$, 795.2891; found, 795.2889.

3.5. *p*-Methoxyphenyl 3-*O*-allyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (6)

To a stirred solution of **4** (81 mg, 91 μmol) in dry MeOH (2.0 mL) was added $NaBH_4$ (10 mg, 0.27 mmol). After stirring for 1 min at 0 °C, the mixture was diluted with CH_2Cl_2, and the reaction mixture was quenched by the addition of water and aq $NaHCO_3$. The organic layer was separated and dried (Na_2SO_4), and the solvent was evaporated. Flash chromatography of the residue gave the β-configured D-mannoside **6** (71 mg, 80 μmol, 87%): $[\alpha]_D^{20}$ +11 (*c* 0.03, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.68–6.68 (23H, m, arom), 5.96–5.86 (1H, m, CH_2CH–CH_2–), 5.63 (1H, d, *J* 6.8 Hz, H-1^{I}), 5.49 (1H, s, benzylidene), 5.31 (1H, dd, *J* 1.4 and 15.6 Hz, CH_2CH–CH_2–), 5.20 (1H, dd, *J* 1.5 and 10.5 Hz, CH_2CH–CH_2–), 4.86 and 4.49 (2H, d, *J* 12.0 Hz, OCH_2Ph), 4.47 and 4.53 (2H, d, *J* 12.9 Hz, OCH_2Ph), 4.73 (1H, s, H-1^{II}), 4.47–4.42 (2H, m, H-2^{I}, 3^{I}), 4.28–3.76 (7H, m , H-5^{I}, 6^{I,II}, CH_2CH–CH_2–), 4.02 (1H, d, *J* 2.0 Hz, H-2^{II}), 3.95 (1H, dd, *J* 8.9 and 9.2 Hz, H-4^{I}), 3.71 (3H, s, OMe), 3.60 (1H, dd, *J* 10.7 and 10.7 Hz, H-4^{II}), 3.43 (1H, dd, *J* 2.0 and 10.7 Hz, H-3^{II}), 3.22–3.17 (1H, m, H-5^{II}), 2.26 (1H, s, OH). HRFABMS (negative-ion mode): Calcd for $C_{51}H_{51}NO_{13}$ $[M]^-$, 885.3360; found, 885.3361.

3.6. *p*-Methoxyphenyl 3-*O*-allyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (7)

3.6.1. From 5. To a stirred solution of **5** (30 mg, 37 μmol) in dry MeOH (1.0 mL) was added $NaBH_4$ (41 mg, 0.11 mmol). After stirring for 1 min at 0 °C, the mixture was diluted with CH_2Cl_2, and the reaction was quenched by the addition of water and aq $NaHCO_3$. The organic layer was separated and dried (Na_2SO_4), and the solvent was evaporated. Flash chromatography of the residue gave **7** (23 mg, 28 μmol, 76%).

3.6.2. From 6. To a solution of compound **6** (120 mg, 0.13 mmol) and ethylene glycol (50 μL) in MeCN (1.0 mL) was added *p*-TsOH·H_2O (27 mg, 0.14 mmol). After stirring for 30 min at room temperature, the reaction was quenched with aq $NaHCO_3$. The mixture was extracted with Et_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and concentrated. Flash chromatography of the residue gave **7** (99 mg, 0.12 mmol, 92%): $[\alpha]_D^{20}$ +12 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.67–6.68 (18H, m, arom), 5.92–5.87 (1H, m, CH_2CH–CH_2–), 5.60 (1H, d, *J* 8.4 Hz, H-1^{I}), 5.28 (1H, dd, *J* 1.7 and 17.2 Hz, CH_2CH–CH_2–), 5.20 (1H, dd, *J* 1.7 and 10.4 Hz, CH_2CH–CH_2–), 4.88 and 4.52 (2H, d, *J* 12.1 Hz, OCH_2Ph), 4.73 and 4.53 (2H, d, *J* 11.9 Hz, OCH_2Ph), 4.66 (1H, s, H-1^{I}), 4.53 (1H, d, *J* 11.9 Hz, OCH_2Ph), 4.45–4.43 (2H, m, H-2^{I}, 3^{I}), 4.19–3.95 (2H, m, CH_2CH–CH_2–), 4.16 (1H, dd, *J* 9.5 and 9.5 Hz, H-4^{I}), 3.99 (1H, d, *J* 2.9 Hz, H-2^{II}), 3.85–3.62 (6H, m, H-4^{II}, 5^{I}, 6^{I,II}), 3.70 (3H, s, OMe), 3.22–3.13 (1H, m, H-5^{II}), 3.15 (1H, dd, *J* 2.9 and 9.7 Hz, H-3^{II}). ^{13}C NMR ($CDCl_3$, 100 MHz, selected signals): δ 99.97 (C-1^{II}), 97.67 (C-1^{I}). HRFABMS (negative-ion mode): Calcd for $C_{44}H_{47}NO_{13}$ $[M]^-$, 797.3047; found, 797.3034.

3.7. *p*-Methoxyphenyl 3-*O*-allyl-6-*O*-*tert*-butyldimethylsilyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (8)

To a solution of **7** (40 mg, 50 μmol) and Et_3N (0.1 mL) in DMF (1.0 mL) was added TBDMSCl (15 mg, 0.10 mmol). After stirring for 30 min at room temperature, the reaction was quenched with aq $NaHCO_3$. The mixture was extracted with Et_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and concentrated. Flash chromatography of the residue gave **8** (39 mg, 43 μmol, 86%): $[\alpha]_D^{20}$ +30 (*c* 0.03, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.67–6.71 (18H, m, arom), 5.99–5.90 (1H, m, CH_2CH–CH_2–), 5.62 (1H, d, *J* 8.3 Hz, H-1^{I}), 5.32–5.20 (2H, m, CH_2CH–CH_2–), 4.86 and 4.49 (2H, d, *J* 12.2 Hz, OCH_2Ph), 4.73 and 4.57 (2H, d, *J* 12.0 Hz, OCH_2Ph), 4.67 (1H, s, H-1^{II}), 4.42–4.38 (2H, m, H-2^{I}, 3^{I}), 4.14–4.06 (3H, m, H-4^{I}, CH_2CH–CH_2–), 3.99 (1H, d, *J* 3.0 Hz, H-2^{II}), 3.84 (3H, s, OMe), 3.86–3.70 (5H, m, H-5^{I}, 6^{I}, 6^{II}), 3.65 (1H, dd, *J* 7.3 and 9.2 Hz, H-4^{II}), 3.21–3.16 (1H, m, H-5^{II}), 3.19 (1H, dd, *J* 3.0 and 9.2 Hz, H-3^{II}), 3.35 (1H, s, OH), 2.37 (1H, s, OH), 0.84 (9H, s, *t*-Bu), 0.04 (3H, s, Me), 0.02 (3H, s, Me). HRFABMS (negative-ion mode): Calcd for $C_{50}H_{61}NO_{13}Si$ $[M]^-$, 911.3912; found, 911.3922.

3.8. *p*-Methoxyphenyl 2,4-di-*O*-acetyl-3-*O*-allyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (9)

To a solution of **8** (20 mg, 22 μmol) in Et_3N (0.5 mL) was added Ac_2O (1.0 mL), and the mixture was stirred for 30 min at room temperature. The mixture was poured into aq $NaHCO_3$, then extracted with Et_2O and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (2.0 mL) and treated with HF·pyridine (0.1 mL) for 30 min at room temperature. The reaction

was quenched with aq $NaHCO_3$. The mixture was extracted with Et_2O, and the organic layer was washed successively with aq $CuSO_4$, and aq $NaHCO_3$ and then concentrated. Flash chromatography of the residue gave **9** (18 mg, 20 μmol, 91%): $[\alpha]_D^{20}$ +35 (*c* 0.06, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.70–6.67 (18H, m, arom), 5.84–5.72 (1H, m, CH_2CH–CH_2–), 5.62 (1H, d, *J* 8.1, H-1^I), 5.35 (1H, d, *J* 3.1, H-2^{II}), 5.36–5.15 (2H, m, CH_2CH–CH_2–), 4.93 (1H, dd, *J* 9.7 and 9.8 Hz, H-4^{II}), 4.89 and 4.46 (2H, d, *J* 11.9 Hz, OCH_2Ph), 4.79 and 4.51 (2H, d, *J* 11.9 Hz, OCH_2Ph), 4.67 (1H, s, H-1^{II}), 4.39 (1H, dd, *J* 8.1 and 9.3 Hz, H-2^I), 4.31 (1H, dd, *J* 8.4 and 9.3 Hz, H-3^I), 4.17 (1H, dd, *J* 8.4 and 9.1 Hz, H-4^I), 4.04 (1H, dd, *J* 5.1 and 13.1 Hz, CH_2CH–CH_2–), 3.71 (3H, s, OMe), 3.84–3.24 (7H, m, $5^{I,II}$, $6^{I,II}$, CH_2CH–CH_2–), 3.30 (1H, dd, *J* 3.1 and 9.7 Hz, H-3^{II}), 2.13 (3H, s, Me), 2.07 (3H, s, Me). HRFABMS (positive-ion mode): Calcd for $C_{48}H_{52}NO_{15}$ $[M+H]^+$, 882.3339; found, 882.3346.

3.9. 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,4-di-*O*-acetyl-3-*O*-allyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranose (11)

A mixture of **9** (50 mg, 57 μmol), **10** (33 mg, 68 μmol) and 4 Å MS (0.10 g) in CH_2Cl_2 (2.0 mL) was stirred for 1 h. $BF_3{\cdot}OEt_2$ (10 μL, 78 μmol) was added to solution at 0 °C. After stirring for 5 min, the reaction was quenched with aq $NaHCO_3$. The mixture was extracted with CH_2Cl_2, and the organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was dissolved in 10:1 CH_3CN–water (2.0 mL) and treated with CAN ($(NH_4)_2Ce(NO_3)_6$) (62 mg, 0.11 mmol) for 30 min at room temperature. The mixture was extracted with Et_2O, and the organic layer was washed with water and brine, dried (Na_2SO_4), and evaporated in vacuo. Flash chromatography of the residue gave **11** (49 mg, 39 μmol, 68%): $[\alpha]_D^{20}$ +36 (*c* 0.05, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.65–6.82 (29H, m, arom), 5.75–5.71 (1H, m, CH_2CH–CH_2–), 5.35 (1H, d, *J* 3.2 Hz, H-2^{II}), 5.34 (1H, s, H-2^{III}), 5.23–5.15 (3H, m, H-1^I, CH_2CH–CH_2–), 5.02 (1H, dd, *J* 9.9 and 9.8 Hz, H-4^{II}), 4.86 (1H, s, H-1^{III}), 4.82 and 4.64 (2H, d, *J* 11.2 Hz, OCH_2Ph), 4.80 and 4.53 (2H, d, *J* 12.2 Hz, OCH_2Ph), 4.79 and 4.43 (2H, d, *J* 12.0 Hz, OCH_2Ph), 4.58 and 4.46 (2H, d, *J* 11.9 Hz, OCH_2Ph), 4.50 and 4.28 (2H, d, *J* 10.5 Hz, OCH_2Ph), 4.78 (1H, s, H-1^{II}), 4.31 (1H, dd, *J* 8.4 and 9.3 Hz, H-3^I), 4.15 (1H, dd, *J* 8.4 and 9.1 Hz, H-4^I), 4.05–3.90 (3H, m, H-2^I, 3^{III}, CH_2CH–CH_2–), 3.85–3.40 (9H, m, H-4^{III}, 5^{III}, $6^{I,II,III}$, CH_2CH–CH_2–), 3.65–3.60 (1H, m, H-5^I), 3.51–3.45 (1H, m, H-5^{II}), 3.24 (1H, dd, *J* 9.9 and 3.2 Hz, H-3^{II}). HRFABMS (negative-ion mode): Calcd for $C_{70}H_{75}NO_{20}$ $[M]^-$, 1249.4882; found, 1249.4901.

3.10. 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,4-di-*O*-acetyl-3-*O*-allyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl fluoride (12)

To a stirred mixture of **11** (49 mg, 39 μmol) in dry CH_2Cl_2 (2.0 mL) was added DAST (0.10 mL, 0.95 mmol). After stirring for 10 min at 0 °C, the reaction was quenched with aq $NaHCO_3$. The mixture was extracted with Et_2O, and the organic layer was washed with aq $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. Flash chromatography of the residue gave **12** (38 mg, 30 μmol, 77%): $[\alpha]_D^{20}$ +35 (*c* 0.13, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz): δ 7.66–6.75 (29H, m, arom), 5.80 (1H, dd, *J* 53.9 and 7.4, H-1^I), 5.74–5.73 (1H, m, CH_2CH–CH_2–), 5.34 (1H, d, *J* 3.0, H-2^{II}), 5.31 (1H, dd, *J* 2.8 and 1.8 Hz, H-2^{III}), 5.20 (1H, dd, *J* 1.5 and 17.5 Hz, CH_2CH–CH_2–), 5.15 (1H, dd, *J* 1.5 and 10.3 Hz, CH_2CH–CH_2–), 5.06 (1H, dd, *J* 9.8 and 9.9 Hz, H-4^{II}), 4.85 (1H, d, *J* 1.8 Hz, H-1^{III}), 4.69 (1H, s, H-1^{II}), 4.81 and 4.51 (2H, d, *J* 11.0 Hz, OCH_2Ph), 4.79 and 4.46 (2H, d, *J* 13.0 Hz, OCH_2Ph), 4.77 and 4.55 (2H, d, *J* 12.3 Hz, OCH_2Ph), 4.60 and 4.45 (2H, d, *J* 12.0 Hz, OCH_2Ph), 4.52 and 4.34 (2H, d, *J* 11.0 Hz, OCH_2Ph), 4.27–4.17 (3H, m, H-2^I, 3^I, 4^I), 4.01 (1H, dd, *J* 5.0 and 13.1 Hz, CH_2CH–CH_2–), 3.90 (1H, dd, *J* 2.8 and 9.3 Hz, H-3^{III}), 3.85 (1H, dd, *J* 9.2 and 9.3 Hz, H-4^{III}), 3.81–3.61 (8H, m, H-5^I, 5^{III}, 6^I, $6a^{II}$, 6^{III}, CH_2CH–CH_2–), 3.55 (1H, dd, *J* 2.9 and 11.5 Hz, H-$6b^{II}$), 3.42–3.39 (1H, m, H-5^{II}), 3.24 (1H, dd, *J* 3.0 and 9.9 Hz, H-3^{II}), 2.14 (3H, s, Ac), 2.05 (3H, s, Ac), 1.99 (3H, s, Ac). ^{13}C NMR ($CDCl_3$, 150 MHz, selected signals): δ 104.87 (J_{CF} 235 Hz, C-1^I), 98.77 (C-1^{II}), 97.84 (C-1^{III}). HRFABMS (negative-ion mode): Calcd for $C_{70}H_{74}FNO_{19}$ $[M]^-$, 1251.4839; found, 1251.4843.

3.11. 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,4-di-*O*-acetyl-3-*O*-allyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl azide (14)

A mixture of acceptor **13** (6.0 mg, 12 μmol), donor **12** (10 mg, 8.0 μmol) and 4 Å MS (0.10 g) in CH_2Cl_2 (1.0 mL) was stirred for 1 h. $BF_3{\cdot}OEt_2$ (5 μL, 39 μmol) was added at 0 °C. After stirring for 5 min, the reaction was quenched with aq $NaHCO_3$. The mixture was extracted with CH_2Cl_2, and the organic layer was washed with brine, dried (Na_2SO_4), and concentrated. Flash chromatography of the residue gave **14** (9.8 mg, 5.6 μmol, 70%): $[\alpha]_D^{20}$ +26 (*c* 0.01, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz): δ 7.77–6.71 (43H, m, arom), 5.79–5.72 (1H, m, CH_2CH–CH_2–), 5.36 (1H, d, *J* 3.0 Hz, H-2^{III}), 5.28 (1H, d, *J* 3.2 Hz, H-2^{IV}), 5.23 (1H, d, *J* 8.3 Hz, H-1^{II}), 5.20 (1H, dd, *J* 1.5 and 17.5 Hz,

$CH_2CH–CH_2–$), 5.14 (1H, dd, J 1.5 and 9.2 Hz, $CH_2CH–CH_2–$), 5.13 (1H, d, J 9.3 Hz, H-1^{I}), 5.10 (1H, dd, J 9.1 and 9.9 Hz, H-4^{III}), 4.81 (1H, s, H-1^{IV}), 4.72 (1H, s, H-1^{III}), 4.82 and 4.45 (2H, d, J 12.8 Hz, OCH_2Ph), 4.81 and 4.46 (2H, d, J 9.5 Hz, OCH_2Ph), 4.77 and 4.42 (2H, d, J 11.1 Hz, OCH_2Ph), 4.61 and 4.52 (2H, d, J 12.3 Hz, OCH_2Ph), 4.59 and 4.41 (2H, d, J 12.2 Hz, OCH_2Ph), 4.51 and 4.49 (2H, d, J 11.8 Hz, OCH_2Ph), 4.48 and 4.30 (2H, d, J 10.9 Hz, OCH_2Ph), 4.25–4.09 (5H, m, H-2^{II}, 3^{I}, 3^{II}, 4^{I}, 4^{II}), 4.02 (1H, dd, J 9.3 and 10.4 Hz, H-2^{I}), 4.04–4.00 (1H, m, $CH_2CH–CH_2–$), 3.86 (1H, dd, J 9.4 and 3.2 Hz, H-3^{IV}), 3.79 (1H, dd, J 9.4 and 9.5 Hz, H-4^{IV}), 3.26 (1H, J 3.0 and 9.9 Hz, H-3^{III}), 3.75–3.25 (13H, m, H-$5^{I,II,III,IV}$, H-$6^{I,II,III,IV}$, $CH_2CH–CH_2–$), 2.17 (3H, s, Ac), 2.01 (3H, s, Ac), 1.91 (3H, s, Ac). ^{13}C NMR ($CDCl_3$, 150 MHz, selected signals): δ 99.20 (C-1^{III}), 97.76 (C-1^{IV}), 96.67 (C-1^{II}), 85.49 (C-1^{I}). HRFABMS (negative-ion mode): Calcd for $C_{98}H_{99}N_5O_{25}$ $[M]^-$, 1745.6644; found, 1745.6644.

Acknowledgements

We thank Professor Minoru Isobe, Nagoya University, for providing helpful discussion. This work was supported in part by a Grant-in-Aid for Scientific Research (Grant No. 11680592) and Grants-in-Aid for Priority Area Research Program (Grant No. 12045256) of the Ministry of Education, Science, Culture, and Sports of Japan.

Supplementary data

NMR spectra for compounds **3**–**11**, **12**, and **14** are provided in Supplementary data, which is available online at doi:10.1016/j.carres.2006.04.015.

References

1. (a) Varki, A. *Glycobiology* **1993**, *3*, 97–130; (b) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683–720; (c) Rudd, P. M.; Elliott, T.; Cresswell, P.; Wilson, I. A.; Dwek, R. A. *Science* **2001**, *291*, 2370–2376; (d) Imperiali, B.; O'Connor, S. E.; Hendrickson, T.; Kellenberger, C. *Pure Appl. Chem.* **1999**, *71*, 777–787; (e) Lis, H.; Sharon, N. *Eur. J. Biochem.* **1993**, *218*, 1–27; (f) Bertozzi, C. R.; Kiessling, L. L. *Science* **2001**, *291*, 2357–2364.
2. (a) Kornfeld, R.; Kornfeld, S. *Annu. Rev. Biochem.* **1985**, *54*, 631–664; (b) Schwarz, R. T.; Datema, R. *Adv. Carbohydr. Chem. Biochem.* **1982**, *40*, 287–379.
3. Tani, N.; Kamada, G.; Ochiai, K.; Isobe, M.; Suwan, S.; Kai, H. *J. Biochem. (Tokyo)* **2001**, *129*, 221–227.
4. (a) Kai, H.; Kotani, Y.; Miao, Y.; Azuma, M. *J. Insect Physiol.* **1995**, *41*, 905–910; (b) Kai, H.; Arai, T.; Yasuda, F. *Chronobiol. Int.* **1999**, *16*, 51–58.
5. Kurahashi, T.; Miyazaki, A.; Murakami, Y.; Suwan, S.; Franz, T.; Isobe, M.; Tani, N.; Kai, H. *Bioorg. Med. Chem.* **2002**, *10*, 1703–1710.
6. Kubelka, V.; Altman, F.; Kornfeld, G.; März, L. *Arch. Biochem. Biophys.* **1994**, *308*, 148–157.
7. Pitchayawasin, S.; Isobe, M.; Tani, N.; Kai, T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2527–2531.
8. Pozsgay, V. Stereoselective Synthesis of β-Mannosides. In *Carbohydrates in Chemistry and Biology. Part 1: Chemistry of Saccharides*; Ernest, B., Hart, G. W., Sinaÿ, P., Eds.; Wiley-VCH: Weinheim, 2000; Vol. 1, pp 319–343.
9. (a) Barresi, F.; Hindsgaul, O. *Can. J. Chem.* **1994**, *72*, 1447–1465; (b) Stork, G.; La Clair, J. L. *J. Am. Chem. Soc.* **1996**, *118*, 247–248; (c) Ito, Y.; Ohnishi, Y.; Ogawa, T.; Nakahara, Y. *Synlett* **1998**, 1102–1104.
10. (a) Dudkin, V. Y.; Crich, D. *Tetrahedron Lett.* **2003**, *44*, 1787–1789; (b) Dudkin, V. Y.; Miller, J. S.; Danishefsky, S. J. *Tetrahedron Lett.* **2003**, *44*, 1791–1793; (c) Dudkin, V. Y.; Miller, J. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 736–738; (d) Mandai, H.; Mukaiyama, T. *Chem. Lett.* **2005**, *34*, 702–703; (e) Tanaka, S.-i.; Takashina, M.; Takimoto, H.; Fujimoto, Y.; Tanaka, K.; Fukase, K. *Synlett* **2005**, *15*, 2325–2328.
11. (a) Matsuo, I.; Isomura, M.; Walson, R.; Ajisawa, K. *Tetrahedron Lett.* **1996**, *37*, 8795–8798; (b) Li, B.; Zeng, Y.; Hauser, S.; Song, H.; Wang, L. X. *J. Am. Chem. Soc.* **2005**, *127*, 9692–9693.
12. (a) Ekborg, G.; Lindberg, B.; Lönngren, J. *Acta Chem. Scand.* **1972**, *26*, 3287–3292; (b) Shaban, M. A.; Jeanloz, R. W. *Carbohydr. Res.* **1976**, *52*, 115–127; (c) Warren, C. D.; Augé, C.; Laver, M. L.; Suzuki, S.; Power, D.; Jeanloz, R. W. *Carbohydr. Res.* **1980**, *82*, 71–83; (d) Kerékgyártó, J.; van der Ven, J. G. M.; Kamerling, J. P.; Lipták, A.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1993**, *238*, 135–145.
13. (a) Lichtenthaler, F. W.; Schneider-Adams, T. *J. Org. Chem.* **1994**, *59*, 6728–6734; (b) Lichtenthaler, F. W.; Lergenmüller, M.; Peters, S.; Varga, Z. *Tetrahedron: Asymmetry* **2003**, *14*, 727–736, and references cited therein.
14. Takeo, K.; Nakaji, T.; Shinmitsu, K. *Carbohydr. Res.* **1984**, *133*, 275–287.
15. Nakano, T.; Ito, Y.; Ogawa, T. *Carbohydr. Res.* **1993**, *243*, 43–69.
16. Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21–73.
17. (a) Ogilvie, K. K.; Shifman, A. L.; Penney, C. L. *Can. J. Chem.* **1979**, *57*, 2230–2238; (b) Kinzy, W.; Schmidt, R. R. *Liebigs Ann. Chem.* **1987**, 407–415.
18. Nicolaou, K. C.; Webber, S. E. *Synthesis* **1986**, 453–461.
19. Ito, Y.; Ogawa, T. *Angew. Chem., Int. Ed. Engl.* **1994**, *17*, 33.
20. Dan, A.; Ito, Y.; Ogawa, T. *J. Org. Chem.* **1995**, *60*, 4680–4681.
21. Matsuo, I.; Nakahara, Y.; Ito, Y.; Nukada, T.; Nakahara, Y.; Ogawa, T. *Bioorg. Med. Chem.* **1995**, *3*, 1455–1463.
22. Inazu, T.; Kobayashi, K. *Synlett* **1993**, 869–870.
23. (a) Miuno, M.; Haneda, K.; Iguchi, R.; Inazu, T. *J. Am. Chem. Soc.* **1999**, *121*, 284–290; (b) Yamamoto, K.; Kadowaki, S.; Watanabe, J.; Kumagai, H. *Biochem. Biophys. Res. Commun.* **1994**, *203*, 244–252; (c) Takegawa, K.; Tabuchi, M.; Yamaguchi, S.; Kondo, A.; Kato, I.; Iwahara, S. *J. Biol. Chem.* **1996**, *270*, 3094–3099; (d) Li, H.; Singh, S.; Zeng, Y.; Song, H.; Wang, L. X. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 895–898.