Carbohydrate Research 388 (2014) 87–93

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Synthesis and plant growth regulation activity of α-D-Man*p*NAc-(1→2)-[α-L-Rha*p*-(1→3)-]α-L-Rha*p*-(1→4)-β-D-Glu*p*NAc-(1→3)-α-L-Rha*p*, the repeating unit of O-antigen of *Rhizobium trifolii 4s*

Guanghui Zong [a], Xiaomei Liang [a], Jianjun Zhang [a,*], Liusheng Duan [b], Weiming Tan [b,*], Daoquan Wang [a]

[a] Department of Applied Chemistry, China Agricultural University, Beijing 100193, China
[b] Engineering Research Centre of Plant Growth Regulators, Ministry of Education, College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, China

ARTICLE INFO

Article history:
Received 18 September 2013
Received in revised form 30 November 2013
Accepted 5 December 2013
Available online 12 December 2013

Keywords:
Synthesis
Oligosaccharide
Rhizobium trifolii 4s
Plant growth regulation activity

ABSTRACT

The synthesis of a pentasaccharide **2** containing acetamido-2-deoxy-D-glucose and acetamido-2-deoxy-D-mannose related to the cell wall polysaccharide of *Rhizobium trifolii 4s* has been achieved by a [2+3] approach from commercially available L-rhamnose, D-glucose, and D-glucosamine as the starting materials. The target molecule was equipped with a *p*-methoxylphenyl handle at the reducing terminus to allow for further glycoconjugate formation via selective cleavage of this group. The bioassay suggested that the synthetic pentasaccharide **2** can stimulate the growth of wheat coleoptile similarly to indole-3-acetic acid (IAA), and promote the wheat seedling development before winter by seed treatment at a concentration of 20 mg/L.

1. Introduction

Overuse of chemical fertilizer to maintain good harvest has posed a threat to our environment and also the country's food security in the past decades. In fact, some plants may not need so much chemical fertilizer, as they and their specific symbiotic bacteria of genus *Rhizobium* can combine nitrogen gas with other elements to form useful nitrogen compounds.[1] The use of chemical fertilizer will be greatly reduced if plants have a good use of biological nitrogen fixation. The lipopolysaccharides (LPSs) of *Rhizobium* are crucial to the bacteroid development and nodule occupancy. Bacteria having defects in LPSs structure also have defects in nodule invasion in plant symbiosis.[2–4] Presenting at the distal part of LPSs, the O-antigenic polysaccharides (OPSs) are in direct contact with the environment, and are closely involved in the process of exchanging signals between rhizobia and legumes.[5] Mutants with LPSs that lack their OPSs or have modified core components either are defective in the formation of infection threads or have the nodules unoccupied.[6–9] With the aim of investigating the biological roles of these OPSs, synthetic studies on the OPSs will be useful. Wang et. al. reported that the structure of OPS of *Rhizobium trifolii 4s* was constituted of a pentasaccharide repeating unit composed of L-rhamnose, *N*-acetyl-D-glucosamine, and *N*-acetyl-D-mannosamine in 3:1:1 molar proportion (Fig. 1, **1**).[10]

To obtain new oligosaccharide resources and discover novel bioactive substances, we reported the first total synthesis of the pentasaccharide repeating unit (Fig. 1, **2**). Moreover, the biological effect of the synthesized pentasaccharide on stimulating plant growth has been determined.

2. Results and discussion

The design of the synthesis of the pentasaccharide **2** is outlined in Scheme 1. Retrosynthetic analysis indicated that the pentasaccharide **2** could be achieved through a convergent strategy involving [2+3] glycosylation of a disaccharide acceptor **20** and a trisaccharide donor **11**. The trisaccharide **11** then could be constructed from the α-(1→2)-linked disaccharide acceptor **8** and the rhamnosyl donor **5**, while the disaccharide acceptor **20** could be built from the rhamnosyl acceptor **13**[11,12] and the glucosaminyl donor **12**.[13]

A number of suitably functionalized monosaccharide intermediates **4**,[14] **5**,[15] **6**,[11,12] **12**,[13] and **13**[11,12] were prepared from the commercially available reducing sugars, such as L-rhamnose, D-glucose, and D-glucosamine, using the previously reported reaction conditions. Compound **4**[14] was prepared in 77% yield from compound **3**[16] using a two-step sequence involving selective de-1-O-aetylation followed by trichloroacetimidate formation (Scheme 2).

* Corresponding authors. Tel.: +86 10 62731115; fax: +86 10 62732219.
E-mail addresses: zhangjianjun@cau.edu.cn (J. Zhang), tanwm@cau.edu.cn (W. Tan).

http://dx.doi.org/10.1016/j.carres.2013.12.006
0008-6215/

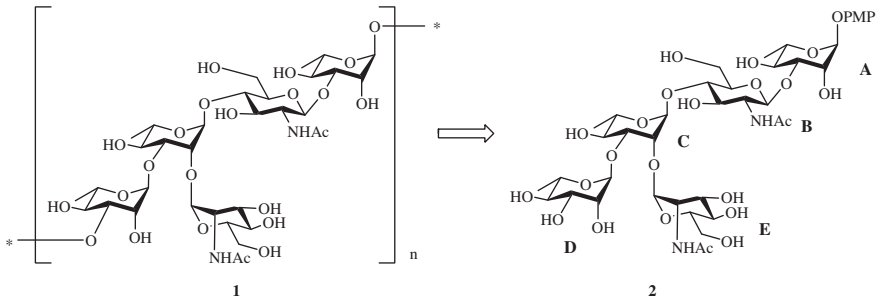


Figure 1. O-Antigenic chain repeating unit of LPSs (**1**) from *Rhizobium trifoki 4s* and the synthesized oligosaccharide **2**.

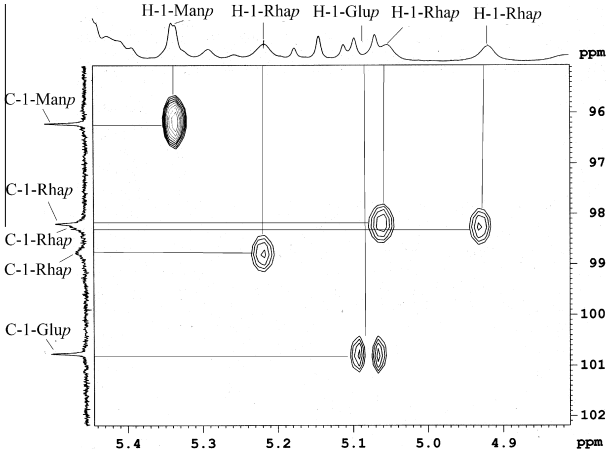
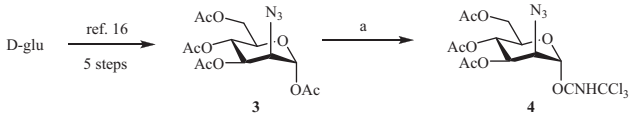
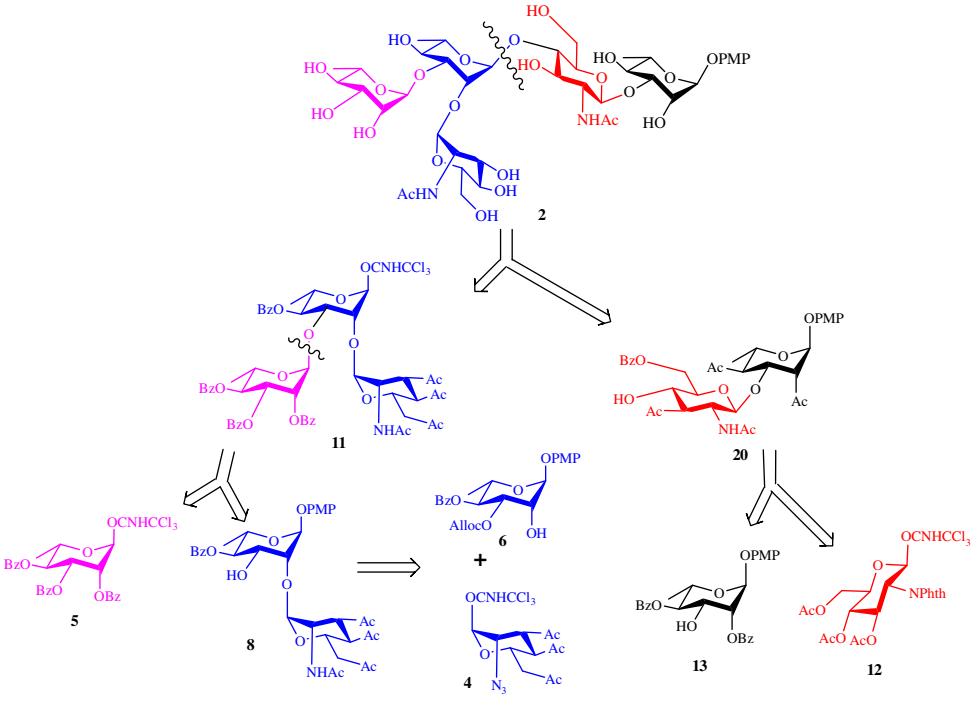


Figure 2. Partial HSQC spectra of the fully protected pentasaccharide **21**.

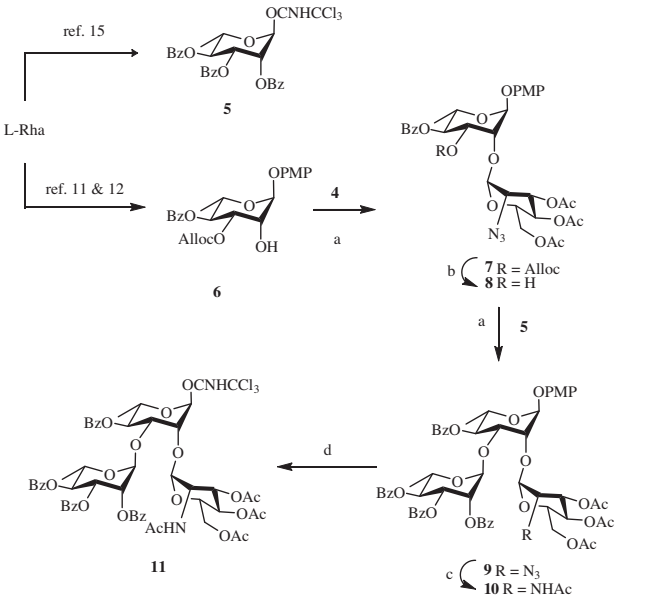


Scheme 2. Synthesis of key synthon **4**. Reagents and conditions: (a) $BnNH_2$, THF, rt, 12 h; then CCl_3CN, DBU, CH_2Cl_2, rt, 0.5 h, 77% over two steps for **4**.

Synthesis of trisaccharide **11** was shown in Scheme 3. Glycosylation between rhamnosyl acceptor **6** and 2-acetamido-2-deoxy-α-D-mannosyl donor **4** was accomplished by using TMSOTf as the catalyst in the presence of 4 Å molecular sieves to afford the disaccharide **7** in 86% yield. Deallyloxycarbonylation of **7** was successfully achieved in MeOH–THF[17] in the presence of CH_3COONH_4, $Pd[P(C_6H_5)_3]_4$, and $NaBH_4$, within 4 min without affecting any of the other protecting groups, giving the desired acceptor **8** in 92% yield. Formation of compound **8** was supported by its spectral analysis [signals at 5.45 ppm (d, J = 1.4 Hz, H-1) and 5.06 ppm (d, J = 1.4 Hz, H-1′) in the ^1H NMR, and 97.4 and 96.3 ppm (2× C-1) in the ^{13}C NMR spectra]. The coupling reaction between trichloroacetimidate **5** and acceptor **8** by using TMSOTf as the catalyst smoothly yielded trisaccharide **9**. The formation of compound **9** was confirmed by its ^{13}C NMR spectrum [signals at 98.7, 96.3 and 95.8 ppm (3× C-1) corresponding to the three anomeric carbons]. Hydrogenolysis of compound **9** followed by N-acetylation



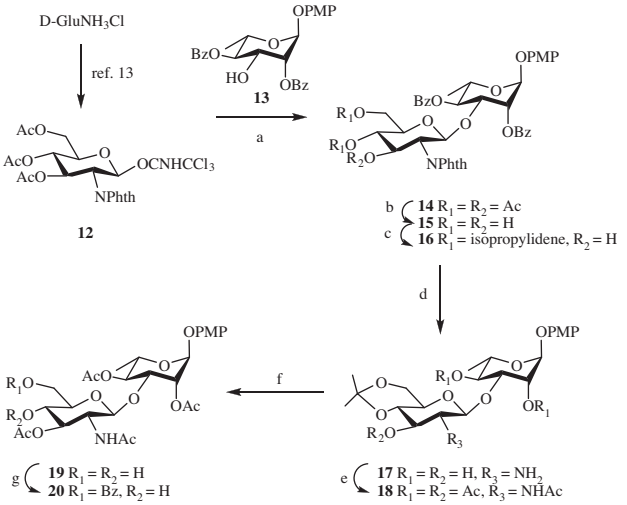
Scheme 1. A retrosynthetic strategy for the synthesis of the target pentasaccharide **2**.



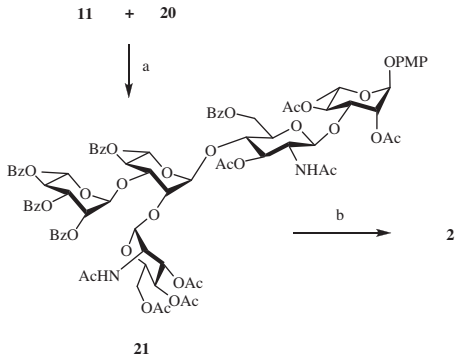
Scheme 3. Synthesis of trisaccharide donor **11**. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, −10 °C to rt, 2 h, 86% for **7**, 75% for **9**; (b) CH_3COONH_4, $Pd[P(C_6H_5)_3]_4$, $NaBH_4$, MeOH–THF, −10 °C, 4 min, 92% for **8**; (c) H_2, Pd/C, 1 atm, 6 h; then Ac_2O, Py, 25 °C, 12 h, 80% for **10**; (d) 4:1 CH_3CN–H_2O, CAN, 30 °C, then CCl_3CN, DBU, CH_2Cl_2, rt, 0.5 h, 68% for two steps for **11**.

produced compound **10** in 80% yield over two steps. The cleavage of the 4-methoxyphenyl group in compound **10** with ceric ammonium nitrate (CAN), followed by reacting with trichloroacetonitrile in the presence of 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU),[18] afforded glycosyl trichloroacetimidate **11**, which was then isolated by flash chromatography on silica gel in 68% yield over two steps.

Having glycosyl donor **11** in hand, our attention was focused on the construction of the disaccharide acceptor **20**, as shown in Scheme 4. The glycosylation of acceptor **13**[11,12] with trichloroacetimidate **12**[13] having a C-2 acetyl ester to control β-anomeric selectivity afforded the corresponding disaccharide **14** in 76% yield. ^1H NMR spectra confirmed its β-linkage formation [signals at



Scheme 4. Synthesis of disaccharide acceptor **20**. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, −10 °C to rt, 2 h, 76% for **14**; (b) AcCl, MeOH, rt, overnight, 90% for **15**; (c) 2,2-dimethyoxypropane, DMF, rt, 2 h, 88% for **16**; (d) 85% $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 3 h; (e) Ac_2O, Py 25 °C,12 h, 79% for **18**; (f) 70% AcOH, 70 °C, 1.5 h, 86 % for **19**; (g) BzCl, Py, −10 °C to rt, 3 h, 81% for **20**.



Scheme 5. Synthesis of the target pentasaccharide **2**. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, −10 °C to rt, 2 h, 69% for **21**; (b) satd NH_3–MeOH, rt, 96 h, 89% for **2**.

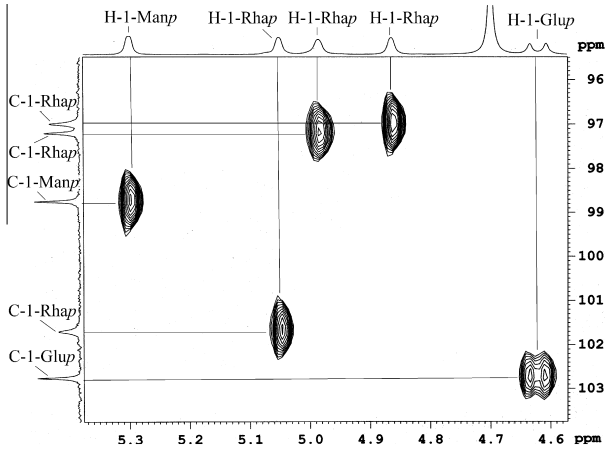


Figure 3. Partial HSQC spectra of the target pentasaccharide **2**.

5.65 ppm (d, 1H, *J* 8.4 Hz, H-1-GluNAc*p*) in the ^1H NMR spectra]. Compound **14** was then subjected to a series of transformations involving (a) selective deacetylation with 2% CH_3COCl–MeOH[19] followed by isopropylidenation with 2,2-dimethyoxypropane in dry DMF in the presence of catalytic $TsOH{\cdot}H_2O$, (b) removal of *N*-phthalimido group using ethylenediamine and EtOH,[20] followed by N-acetylation, (c) deisopropylidenation in 70% AcOH and selective benzoylation of the 6-OH of D-glucose moiety to furnish compound **20** in 23% overall yield over seven steps. The structure of **20** was confirmed by its ^1H and ^{13}C NMR spectra, showing the characteristic signals, such as 5.33 ppm (d, 1H, *J* 1.8 Hz, H-1-Rha*p*), 4.92 ppm (d, 1H, *J* 8.2 Hz, H-1-GluNAc*p*), and 100.8 (C-1), 96.3 ppm (2× C-1).

Finally, the synthesis of the target pentasaccharide **2** was shown in Scheme 5. The condensation of **11** with the acceptor **20** by following the same glycosylation strategy as mentioned above gave the fully protected pentasaccharide **21** in 69% yield. The presence of the five anomeric carbon signals in the ^{13}C NMR spectra confirmed its formation [100.8, 98.8, 98.3, 98.2, and 96.2 ppm]. The deacylation of the pentasaccharide **21** with ammonia-saturated methanol afforded the target pentasaccharide **2** (89%). The complete assignment of the ^1H and ^{13}C signals of **21** and **2** was achieved by the HSQC experiments (Figs. 2 and 3).

2.1. Effects on the growth of wheat plants

The results of the growth regulation activity tests given in Tables 1 and 2 clearly show that the synthetic pentasaccharide **2** promoted the elongation of wheat coleoptile and also

Table 1
Plant growth promotion activity data of synthetic pentasaccharide for wheat coleoptile

Concentration (mg/L)	β-Indoleacetic acid, IAA		Synthetic pentasaccharide **2**	
	Coleoptile length (mm)	Promotion rate (%)	Coleoptile length (mm)	Promotion rate (%)
0	6.47 ± 0.24	/	6.47 ± 0.24	/
0.01	7.16 ± 0.31	+27.9	6.95 ± 0.32	+19.4
0.1	7.81 ± 0.35	+54.3	8.03 ± 0.38	+63.2
1.0	8.43 ± 0.38	+79.4	8.66 ± 0.41	+88.7

Table 2
Wheat seedling growth morphological characters on 49th day after planting, using the synthetic pentasaccharide **2** for seed treatment

Treatments	Control (tap water)	Synthetic pentasaccharide **2**, 20 mg/L
Expansion leaves number of the stem	6.0 ± 0.3	7.3 ± 0.4
Till number	4.9 ± 0.3	6.2 ± 0.4
Till number with no less than 3 leaves	1.5 ± 0.1	2.5 ± 0.2
Total root number	11.2 ± 0.7	13.1 ± 0.6

stimulated the growth of wheat seedlings. For wheat coleoptile growth (Table 1), the promotion effects of 0.1 to 1.0 mg/L synthetic pentasaccharide exceeded the control compound IAA. When used for the wheat seed treatment (Table 2), the number of the expansion leaves on the main stem, the till number, the till number with no less than 3 leaves and the total root number of each plant were all higher than those of the control seedlings, indicating that the stimulation effects of the synthetic pentasaccharide were evident.

3. Conclusion

In conclusion, a pentasaccharide analog (**2**), related to the repeating unit of the O-antigenic polysaccharide of *Rhizobium trifoki 4s*, has been synthesized using a [2+3] block glycosylation strategy for the first time. All intermediates were obtained in relatively high yield. Also, we presented the first report regarding the plant growth activity of this O-antigenic polysaccharide. By our bioassay studies, we found the synthetic pentasaccharide could promote wheat coleoptile growth at low concentration (<1 mg/L), and had the similar growth promotion effect compared to the known plant harmony IAA. Moreover, we demonstrated that the synthetic pentasaccharide **2** could be used as a wheat seed treatment agent to gain flourishing seedlings before winter.

4. Experimental procedures

4.1. General methods

Optical rotations were determined with a Perkin–Elmer model 241-MC automatic polarimeter for solutions in a 1-dm, jacketed cell. ^{1}H and ^{13}C NMR spectra were recorded with Bruker DPX300 and Bruker AVANCE600 spectrometers in $CDCl_3$ or D_2O solutions. Internal references: TMS (δ 0.000 ppm for ^{1}H), $CDCl_3$ (δ 77.00 ppm for ^{13}C), HOD (δ 4.700 for ^{1}H). Elemental analysis was performed on a Yanaco CHN Corder MF-3 automatic elemental analyzer. High-resolution mass spectra (HRMS) were acquired by the Peking University, and electrospray-ionization mass spectra (ESIMS) were acquired by the China Agricultural University. Thin-layer chromatography (TLC) was performed on silica gel HF with detection by charring with 30% (v/v) H_2SO_4 in MeOH or by UV. Column chromatography was conducted by elution of a column of silica gel (200–300 mesh) with EtOAc/petroleum ether (bp 60–90 °C) as the eluent. Solutions were concentrated at temperature <60 °C under diminished pressure.

4.2. Synthesis

4.2.1. *p*-Methoxyphenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranosyl-(1→2)-3-*O*-allyloxycarbonyl-4-*O*-benzoyl-α-L-rhamnopyranoside (7)

Compound **6** (3.6 g, 7.9 mmol), **4** (4.1 g, 8.6 mmol), and 4 Å molecular sieves (4 g) were dried together under high vacuum for 2 h, then dissolved in anhydrous, redistilled CH_2Cl_2 (100 mL). TMSOTf (54 μL, 0.3 mmol) was added dropwise at −10 °C under an N_2 atmosphere. The reaction mixture was stirred for 0.5 h, during which time the mixture was allowed to gradually warm to ambient temperature. TLC (3:6:1 petroleum ether–toluene–EtOAc) indicated that the reaction was complete. Then the reaction mixture was neutralized with triethylamine and filtrated, and the filtrate was concentrated. Purification of the residue by column chromatography (4:6:1 petroleum ether–toluene–EtOAc) gave **7** (5.2 g, 86%) as a white solid. $[\alpha]_D^{25} + 55.9°$ (*c* 0.3 $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.04–8.02 (m, 2H, Bz-*H*), 7.57 (m, 1H, Bz-*H*), 7.47–7.42 (m, 2H, Bz-*H*), 7.06–7.03 (m, 2H, Ar-*H*), 6.87–6.84 (m, 2H, Ar-*H*), 5.69 (m, 1H, CH_2═*CH*CH_2OCO), 5.53 (dd, 1H, $J_{2',3}$ 3.4 Hz, $J_{3',4'}$ 10.1 Hz, H-3′), 5.46–5.37 (m, 4H, H-1′, H-3, H-4, H-4′), 5.23–5.06 (m, 2H, *C*H_2═CHCH_2OCO), 4.96 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1), 4.52–4.49 (m, 2H, CH_2═CH*C*H_2OCO), 4.36 (dd, 1H, $J_{1',2'}$ 2.0 Hz, $J_{2',3'}$ 3.3 Hz, H-2′), 4.32–4.23 (m, 2H, H-5, H-5′), 4.22 (dd, 1H, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 3.2 Hz, H-2), 4.17–4.02 (m, 2H, 2× H-6′), 2.12, 2.09, 2.07 (3s, 9H, 3× *C*H_3CO), 1.28 (d, 1H, *J* 6.3 Hz, H-6). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.7, 169.7, 169.6 (3× *C*OCH$_3$), 165.4 (*C*OPh), 165.4, 154.2, 130.8, 119.2 (*C*H_2═*C*HCH_2OCO), 97.6, 96.2 (2× C-1), 55.6 ($C_6H_4OCH_3$), 20.6(2), 20.4 (3× *C*OCH$_3$), 17.5 (C-6). ESI-MS *m*/*z* calcd for $C_{36}H_{41}N_3NaO_{16}$ $(M+Na)^+$ 794.2. Found: 793.9. Anal. Calcd for $C_{36}H_{41}N_3O_{16}$: C, 56.03; H, 5.35. Found: C, 56.22; H, 5.31.

4.2.2. *p*-Methoxyphenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranosyl-(1→2)-4-*O*-benzoyl-α-L-rhamnopyranoside (8)

To a cooled (−5 °C) solution of compound **7** (4.0 g, 5.2 mmol) in 1:1 MeOH–THF (100 mL) in 500 mL flask was added CH_3COONH_4 (4.0 g, 52 mmol). With vigorous stirring, $NaBH_4$ (47 mg, 1.3 mmol), $Pd[P(C_6H_5)_3]_4$ (240 mg, 0.21 mmol), and $NaBH_4$ (234 mg, 6.5 mmol) were added in 3 portions immediately one after another. 1 min after the addition of the second portion of $NaBH_4$, TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was concentrated under diminished pressure, the residue was dissolved in CH_2Cl_2 (150 mL) and washed with water (50 mL), then the organic phase was dried over Na_2SO_4. Evaporation and purification by flash column chromatography (petroleum ether–EtOAc 3:1) afforded compound **8** as a white foam

(3.3 g, 92%). $[\alpha]_D^{25}$ + 2.4° (*c* 0.6 $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 8.07–8.05 (m, 2H, Bz-*H*), 7.58 (m, 1H, Bz-*H*), 7.48–7.43 (m, 2H, Bz-*H*), 7.05–7.01 (m, 2H, Ar-*H*), 6.87–6.83 (m, 2H, Ar-*H*), 5.48 (dd, 1H, $J_{2',3'}$ 3.8 Hz, $J_{3',4'}$ 9.4 Hz, H-3′), 5.45 (d, 1H, $J_{1,2}$ 1.4 Hz, H-1), 5.34 (t, 1H, $J_{3',4'}$, $J_{4',5'}$ 9.4 Hz, H-4′), 5.13 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.7 Hz, H-4), 5.06 (d, 1H, $J_{1',2'}$ 1.4 Hz, H-1′), 4.39 (m, 1H, H-3), 4.33–4.26 (m, 2H, H-5, H-5′), 4.24 (dd, 1H, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 3.4 Hz, H-2), 4.19 (dd, 1H, $J_{1',2'}$ 2.0 Hz, $J_{2',3'}$ 3.8 Hz, H-2′), 4.15–4.08 (m, 2H, 2× H-6′), 3.10 (d, 1H, *J* 8.8 Hz, O*H*), 2.12, 2.09, 2.05 (3s, 9H, 3× CH_3CO), 1.27 (d, 1H, *J* 6.2 Hz, H-6). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.7, 169.9, 169.5 (3× *C*OCH$_3$), 166.9 (*C*OPh), 155.2, 150.2, 117.6(2), 114.7(2) ($C_6H_4OCH_3$), 97.4, 96.3 (2× C-1), 55.6 ($C_6H_4OCH_3$), 20.6(2), 20.5 (3× *C*OCH$_3$), 17.5 (C-6). ESI-MS *m*/*z* calcd for $C_{32}H_{37}N_3NaO_{14}$ $(M+Na)^+$ 710.2. Found: 709.9. Anal. Calcd for $C_{32}H_{37}N_3O_{14}$: C, 55.89; H, 5.42; N, 6.11. Found: C, 55.70; H, 5.36; N, 6.23.

4.2.3. *p*-Methoxyphenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranosyl-(1→2)-[2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-]4-*O*-benzoyl-α-L-rhamnopyranoside (9)

Compound **8** (2.9 g, 4.2 mmol) and **5** (2.9 g, 4.7 mmol) were coupled in the presence of catalytic TMSOTf (54 μL, 0.3 mmol) under the same conditions as described above for the coupling of **6** with **4**. Purification by silica gel chromatography with 4:4:1 petroleum ether–toluene–EtOAc as the eluent gave trisaccharide **9** (3.6 g, 75%) as a foamy solid. $[\alpha]_D^{25}$ + 101.8° (*c* 0.5 $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 8.04–7.17 (m, 20H, Bz-*H*), 7.08–7.04 (m, 2H, Ar-*H*), 6.88–6.84 (m, 2H, Ar-*H*), 5.62–5.60 (m, 2H, 2× H-3), 5.60–5.49 (m, 5H, H-1, H-2, 3× H-4), 5.34 (br, 1H, H-1), 5.16 (br, 1H, H-1), 4.75–4.55 (m, 2H, 2× H-2), 4.50–4.00 (m, 6H, H-3, 3× H-5, 2× H-6), 3.80 (s, 1H, $C_6H_4OCH_3$), 2.11, 2.10, 2.06 (3s, 9H, 3× CH_3CO), 1.42 (d, 1H, *J* 6.2 Hz, H-6), 1.29 (d, 1H, *J* 6.0 Hz, H-6). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4, 170.0, 170.5 (3× *C*OCH$_3$), 165.9, 165.2, 164.9, 164.5 (4× *C*OPh), 155.4, 150.2, 117.8(2), 114.7(2) ($C_6H_4OCH_3$), 98.7, 96.3, 95.8 (3× C-1), 55.6 ($C_6H_4OCH_3$), 20.6(2), 20.4 (3× *C*OCH$_3$), 17.7, 17.6 (2× C-6). ESI-MS *m*/*z* calcd for $C_{59}H_{59}N_3NaO_{21}$ $(M+Na)^+$ 1168.3. Found: 1168.1. Anal. Calcd for $C_{59}H_{59}N_3O_{21}$: C, 61.83; H, 5.19; N, 3.67. Found: C, 62.11; H, 5.20; N, 3.78.

4.2.4. *p*-Methoxyphenyl 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-mannopyranosyl-(1→2)-[2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-]4-*O*-benzoyl-α-L-rhamnopyranoside (10)

A mixture of compound **9** (1.5 g, 1.3 mmol) and Pd/C (0.25 g) in methanol (100 mL) was stirred under 1 atm of H_2 at rt for 4 h. TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was filtered and the filtrate was concentrated under diminished pressure, and the residue was used for next step directly without purification. A solution of the residue in pyridine (10 mL) and Ac_2O (3 mL) was stirred at rt for 12 h, and TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was concentrated, and then the residue was purified by flash column chromatography on a silica gel column (2:1 petroleum ether–EtOAc) to give trisaccharide **10** (1.2 g, 80%) as a white foamy solid. $[\alpha]_D^{25}$ + 81.6° (*c* 0.7 $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 8.04–7.17 (m, 20H, Bz-*H*), 7.08–7.05 (m, 2H, Ar-*H*), 6.88–6.84 (m, 2H, Ar-*H*), 5.91 (d, 1H, *J* 8.2 Hz, N*H*), 5.68 (dd, 1H, $J_{2,3}$ 3.1 Hz, $J_{3,4}$ 10.2 Hz, H-3), 5.62-5.48 (m, 5H, H-1, H-2-Rha*p*, H-3, 2× H-4), 5.35–5.25 (m, 2H, H-1, H-4), 5.18 (br, 1H, H-1), 4.77 (m, 1H, H-2-Man*p*), 4.67–4.38 (m, 3H, H-2, H-3, H-5), 4.38–4.03 (m, 4H, H-5, 2× H-6), 2.14, 2.03, 2.01 (3s, 9H, 3× CH_3COO), 2.08 (s, 3H, CH_3CONH), 1.41 (d, 1H, *J* 5.9 Hz, H-6-Rha*p*), 1.29 (d, 1H, *J* 6.0 Hz, H-6-Rha*p*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4, 170.2, 169.5 (3× *C*OCH$_3$), 165.8, 165.2, 165.0, 164.5 (4× *C*OPh), 155.2, 150.0, 117.6(2), 114.7(2) ($C_6H_4OCH_3$), 98.8, 98.1, 96.0 (3× C-1), 55.6 ($C_6H_4OCH_3$), 23.2 (NHCOCH$_3$), 20.7(2), 20.6 (3× *C*OCH$_3$), 17.7, 17.6 (2× C-6). ESI-MS *m*/*z* calcd for $C_{61}H_{63}NNaO_{22}$ $(M+Na)^+$ 1184.4. Found: 1184.5. HRMS for $C_{61}H_{64}NO_{22}$ $(M+H)^+$ 1162.3920. Found: 1162.3897.

4.2.5. 3,4,6-Tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-mannopyranosyl-(1→2)-[2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-]4-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (11)

To a solution of compound **10** (1.1 g, 0.95 mmol) in acetonitrile (16 mL) and water (4 mL) was added ceric ammonium nitrate (CAN) (2.1 g, 3.8 mmol). The mixture was stirred for 20 min at 30 °C, at the end of which time TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete. The solvent was evaporated under diminished pressure at 50 °C to give a residue, which was diluted with CH_2Cl_2, and washed with water. The organic phase was dried and concentrated. Purification by silica gel chromatography with 2:1 petroleum ether–EtOAc as the eluent afforded 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-mannopyranosyl-(1→2)-[2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-]4-*O*-benzoyl-α-L-rhamnopyranoside. A mixture of this compound, trichloroacetonitrile (0.5 mL, 5 mmol), and 1,8-diazabicyclo[5.4.0]undecene (DBU) (0.05 mL, 0.4 mmol) in dry CH_2Cl_2 (30 mL) was stirred for 0.5 h and then concentrated. The residue was purified by chromatography with 2:1 petroleum ether–EtOAc as the eluent to give **11** (0.75 g, 68%) as a white foam. $[\alpha]_D^{25}$ + 98.3° (*c* 0.6 $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 8.72 (s, 1H, CN*H*CCl$_3$), 8.04–7.17 (m, 20H, Bz-*H*), 6.41 (d, 1H, $J_{1,2}$ 1.6 Hz, H-1), 5.85 (d, 1H, *J* 8.2 Hz, N*H*), 5.75–5.45 (m, 6H, H-1, H-2, 2× H-3, 2× H-4), 5.33 (t, 1H, *J* 9.9 Hz, H-4), 5.23 (br, 1H, H-1), 4.80 (m, 1H, H-2-Man*p*), 4.70 (m, 1H, H-3), 4.57–4.53 (m, 2H, H-2, H-5), 4.50–4.00 (m, 4H, 2× H-5, 2× H-6), 2.15 (s, 3H, CH_3COO), 2.10 (s, 3H, CH_3CONH), 2.04(2) (s, 6H, 2× CH_3COO), 1.35 (d, 1H, *J* 6.5 Hz, H-6-Rha*p*), 1.33 (d, 1H, *J* 6.3 Hz, H-6-Rha*p*). ESI-MS *m*/*z* calcd for $C_{56}H_{57}Cl_3N_2NaO_{21}$ $(M+Na)^+$ 1221.2. Found: 1221.3. Anal. Calcd for $C_{56}H_{57}Cl_3N_2O_{21}$: C, 56.03; H, 4.79; N, 2.33. Found: C, 56.24; H, 4.58; N, 2.08.

4.2.6. *p*-Methoxyphenyl 3,4,6-tri-*O*-acetyl-2-phthalimido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzoyl-α-L-rhamnopyranoside (14)

Compound **13** (2.4 g, 5.0 mmol) and **12** (3.2 g, 5.5 mmol) were coupled in the presence of catalytic TMSOTf (54 μL, 0.3 mmol) under the same conditions as described above for the coupling of **6** with **4**. Purification by silica gel chromatography with 2:1 petroleum ether–EtOAc as the eluent gave disaccharide **14** (3.4 g, 76%) as a foamy solid. $[\alpha]_D^{25}$ + 9.6° (*c* 0.5 $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 8.13–7.16 (m, 14H, Bz-*H*, Phth-*H*), 7.06–7.03 (m, 2H, Ar-*H*), 5.67 (m, 1H, H-2), 5.65 (d, 1H, *J* 8.4 Hz, H-1′), 5.64 (t, 1H, $J_{2',3'}$, $J_{3',4'}$ 10.6 Hz, H-3′), 5.56 (d, 1H, *J* 1.6 Hz, H-1), 5.43 (t, 1H, *J* 9.8 Hz, H-4), 5.03 (t, 1H, *J* 9.9 Hz, H-4′), 5.49 (dd, 1H, $J_{2,3}$ 3.7 Hz, $J_{3,4}$ 9.7 Hz, H-3), 4.26 (dd, 1H, $J_{1',2'}$ 8.4 Hz, $J_{2',3'}$ 10.7 Hz, H-2′), 4.20–4.11 (m, 2H, 2× H-6′), 4.04, 3.89 (2 m, 2H, 2× H-5), 3.78 (s, 1H, $C_6H_4OCH_3$), 1.96, 1.88, 1.72 (3s, 9H, 3× CH_3CO), 1.09 (d, 1H, *J* 6.2 Hz, H-6). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6, 169.9, 169.2, 165.9, 164.7 (5× *C*O), 155.2, 150.0, 117.7(2), 114.6(2) ($C_6H_4OCH_3$), 98.5, 96.3 (2× C-1), 72.3, 71.9, 71.5, 70.4, 68.5, 66.8, 61.7, 55.5 ($C_6H_4OCH_3$), 54.5, 20.4(2), 20.2 (3× *C*H$_3$CO), 17.4 (C-6-Rha*p*). ESI-MS *m*/*z* calcd for $C_{47}H_{45}NNaO_{17}$ $(M+Na)^+$ 918.2. Found: 918.1. Anal. Calcd for $C_{47}H_{45}NO_{17}$: C, 63.01; H, 5.06; N, 1.56. Found: C, 62.79; H, 5.21; N, 1.40.

4.2.7. *p*-Methoxyphenyl 2-phthalimido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzoyl-α-L-rhamnopyranoside (15)

To a solution of compound **14** (1.8 g, 2.0 mmol) in anhyd MeOH (40 mL) was added AcCl (0.40 mL). The reaction mixture was stirred at rt overnight until which time TLC (1:2 petroleum ether–EtOAc) showed that the starting material had disappeared. The solution was neutralized with Et_3N, then concentrated to dryness.

The residue was passed through a short silica gel column (1:1 petroleum ether–EtOAc) to give **15** (1.4 g, 90%) as a white solid. $[\alpha]_D^{25}$ − 17.0° (*c* 0.9 $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 8.11–7.10 (m, 14H, Bz-*H*, Phth-*H*), 7.06–7.03 (m, 2H, Ar-*H*), 6.85–6.82 (m, 2H, Ar-*H*), 5.87 (dd, 1H, $J_{1,2}$ 1.8 Hz, $J_{2,3}$3.5 Hz, H-2), 5.46 (d, 1H, *J* 8.2 Hz, H-1′), 5.42 (d, 1H, *J* 1.6 Hz, H-1), 5.38 (t, 1H, *J* 9.7 Hz, H-4), 4.42 (dd, 1H, $J_{2,3}$ 3.7 Hz, $J_{3,4}$ 9.7 Hz, H-3), 4.11–4.01 (m, 2H, 2× H-5), 3.92–3.82 (m, 2H, H-3′, O*H*), 3.77 (s, 1H, $C_6H_4OCH_3$), 3.61 (m, 1H, H-2′), 3.46 (m, 1H, H-4′), 3.38–3.30 (m, 2H, 2× H-6′), 3.24 (t, 1H, *J* 7.5 Hz, O*H*), 2.98 (d, 1H, *J* 5.8 Hz, O*H*), 1.09 (d, 1H, *J* 6.2 Hz, H-6). ESI-MS *m*/*z* calcd for $C_{41}H_{39}NNaO_{14}$ $(M+Na)^+$ 792.2. Found: 792.0. Anal. Calcd for $C_{41}H_{39}NO_{14}$: C, 63.97; H, 5.11; N, 1.82. Found: C, 64.28; H, 5.30; N, 1.87.

4.2.8. *p*-Methoxyphenyl 4,6-*O*-isopropylidene-2-phthalimido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzoyl-α-L-rhamnopyranoside (16)

To a solution of compound **15** (1.3 g, 1.7 mmol) in anhyd DMF (20 mL) was added $TsOH{\cdot}H_2O$ (6.4 mg, 0.034 mmol) and 2-methoxypropene (0.31 mL, 2.5 mmol) under a N_2 atmosphere. The mixture was stirred at rt for 3 h, at the end of which time TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete, then concentrated, and co-evaporated with toluene for two times to give a residue that was purified by column chromatography (2:1 petroleum ether–EtOAc) to give **16** (1.2 g, 88%) as a white foam. $[\alpha]_D^{25}$ − 25.0° (*c* 0.6 $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 8.14–7.21 (m, 14H, Bz-*H*, Phth-*H*), 7.06–7.03 (m, 2H, Ar-*H*), 6.85–6.82 (m, 2H, Ar-*H*), 5.59 (dd, 1H, $J_{1,2}$ 1.9 Hz, $J_{2,3}$ 3.6 Hz, H-2-Rha*p*), 5.53 (d, 1H, *J* 1.8 Hz, H-1-Rha*p*), 5.45 (d, 1H, *J* 8.2 Hz, H-1-Glu*p*), 5.40 (t, 1H, *J* 9.8 Hz, H-4-Rha*p*), 4.48 (dd, 1H, $J_{2,3}$ 3.6 Hz, $J_{3,4}$ 9.7 Hz, H-3-Rha*p*), 4.29 (m, 1H, H-2-Glu*p*), 4.12–3.91 (m, 3H, H-3-Glu*p*, H-4-Glu*p*, H-5), 3.77 (s, 1H, $C_6H_4OCH_3$), 3.59 (m, 1H, H-5), 3.47–3.42 (m, 2H, 2× H-6-Glu*p*), 2.14 (d, 1H, *J* 3.6 Hz, O*H*), 1.40, 1.37 (2s, 6H, Me_2C), 1.08 (d, 1H, *J* 6.3 Hz, H-6). ESI-MS *m*/*z* calcd for $C_{44}H_{43}NNaO_{14}$ $(M+Na)^+$ 832.2. Found: 831.9. Anal. Calcd for $C_{44}H_{43}NO_{14}$: C, 65.26; H, 5.35; N, 1.73. Found: C, 65.41; H, 5.14; N, 1.55.

4.2.9. *p*-Methoxyphenyl 3-*O*-acetyl-4,6-*O*-isopropylidene-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranoside (18)

A solution of compound **16** (1.1 g, 1.4 mmol) in 85% $NH_2NH_2{\cdot}H_2O$ (4 mL) and EtOH (36 mL) was heated at 90 °C for 48 h. The reaction mixture was cooled and concentrated and co-evaporated with toluene for two times to give **17** as a syrup. The syrup was then dissolved in pyridine (10 mL) and Ac_2O (3 mL). The mixture was stirred at rt for 12 h at the end of which time TLC (1:2 petroleum ether–EtOAc) indicates that the reaction was complete. The solvents were concentrated and then co-evaporated with toluene for two times to give a residue that was purified by column chromatography (1:1 petroleum ether–EtOAc) to give compound **18** (0.69 g, 79%) as a white foam. $[\alpha]_D^{25}$ − 3.8° (*c* 0.3 $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 6.98–6.93 (m, 2H, Ar-*H*), 6.84–6.80 (m, 2H, Ar-*H*), 5.72 (m, 1H, N*H*Ac), 5.32–5.30 (m, 2H, H-1-Rha*p*, H-2-Rha*p*), 5.14 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.6 Hz, H-4-Rha*p*), 5.10 (t, 1H, $J_{2',3'}$, $J_{3',4'}$ 9.8 Hz, H-3-Glu*p*), 4.71 (d, 1H, *J* 8.2 Hz, H-1-Glu*p*), 4.22 (dd, 1H, $J_{2,3}$ 2.8 Hz, $J_{3,4}$ 9.6 Hz, H-3-Rha*p*), 3.37-3.77 (m, 7H, H-2-Glu*p*, 2× H-6-Glu*p*, H-5, $C_6H_4OCH_3$), 3.71 (t, 1H, $J_{3',4'}$, $J_{4',5'}$ 9.6 Hz, H-4-Glu*p*), 2.16, 2.10, 2.07, 1.95 (4s, 12H, 4× CH_3CO), 1.47, 1.38 (2s, 6H, Me_2C). ESI-MS *m*/*z* calcd for $C_{30}H_{41}NNaO_{14}$ $(M+Na)^+$ 662.2. Found: 661.9. Anal. Calcd for $C_{30}H_{41}NO_{14}$: C, 56.33; H, 6.46; N, 2.19. Found: C, 56.14; H, 6.28; N, 2.17.

4.2.10. *p*-Methoxyphenyl 3-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranoside (19)

Compound **18** (0.62 g, 0.97 mmol) was dissolved in 70% AcOH (20 mL) and stirred at 75 °C for 1.5 h, at the end of which time TLC (1:4 petroleum ether–EtOAc) indicated completion of the reaction. The mixture was concentrated under diminished pressure and then co-evaporated with toluene for two times. The residue was passed through a short silica gel column with 1:3 petroleum ether–EtOAc as the eluent to give **19** (0.50 g, 86%) as a white solid. $[\alpha]_D^{25}$ − 40.2° (*c* 0.4 $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 6.98 (d, 2H, *J* 9.1 Hz, Ar-*H*), 6.82 (d, 2H, *J* 9.1 Hz, Ar-*H*), 6.12 (d, 1H, *J* 8.6 Hz, N*H*Ac), 5.55 (dd, 1H, $J_{1,2}$ 1.8 Hz, $J_{2,3}$ 3.4 Hz, H-2-Rha*p*), 5.27 (d, 1H, *J* 1.5 Hz, H-1-Rha*p*), 5.20 (t, 1H, $J_{2',3'}$, $J_{3',4'}$ 9.3 Hz, H-3-Glu*p*), 5.08 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.7 Hz, H-4-Rha*p*), 4.91 (d, 1H, *J* 8.2 Hz, H-1-Glu*p*), 4.19 (dd, 1H, $J_{2,3}$ 3.5 Hz, $J_{3,4}$ 9.7 Hz, H-3-Rha*p*), 3.94–3.89 (m, 3H, 2× H-6-Glu*p*, H-2-Glu*p*), 3.77 (s, 3H, $C_6H_4OCH_3$), 3.66–3.63 (m, 3H, 2× O*H*, H-4-Glu*p*), 3.51 (m, 1H, H-5), 3.33 (m, 1H, H-5), 2.18, 2.10, 2.08, 1.94 (4s, 12H, 4× CH_3CO), 1.16 (d, 3H, *J* 6.2 Hz, H-6-Rha*p*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.9, 171.3, 170.4, 169.8 (4× Me*C*O), 155.2, 149.7, 117.7(2), 114.6(2) ($C_6H_4OCH_3$), 101.1, 96.4 (2× C-1), 75.8, 75.1, 72.0, 71.2, 68.6, 68.5, 66.8, 55.6 ($C_6H_4OCH_3$), 55.0, 23.2, 21.1, 20.9(2) (4× CH_3CO), 17.4 (C-6-Rha*p*). ESI-MS *m*/*z* calcd for $C_{27}H_{37}NNaO_{14}$ $(M+Na)^+$ 622.2. Found: 622.2. Anal. Calcd for $C_{27}H_{37}NO_{14}$: C, 54.09; H, 6.22; N, 2.34. Found: C, 54.13; H, 6.06; N, 2.17.

4.2.11. *p*-Methoxyphenyl 3-*O*-acetyl-4-*O*-benzoyl-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranoside (20)

To a solution of compound **19** (0.38 g, 0.63 mmol) in pyridine (5 mL) was added dropwise a solution of benzoyl chloride (0.081 mL, 0.70 mmol) in pyridine (3 mL) over 30 min at −10 °C. The reaction mixture was slowly raised to rt and stirred for a further 2 h, at the end of which time TLC (1:4 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was concentrated, and purification of the residue by column chromatography on silica gel (1:2 petroleum ether–EtOAc) gave **20** (0.36 g, 81%) as a white foam. $[\alpha]_D^{25}$ − 52.1° (*c* 0.8 $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 8.08–8.05 (m, 2H, Bz-*H*), 7.51 (m, 1H, Bz-*H*), 7.38–7.33 (m, 2H, Bz-*H*), 6.89 (d, 2H, *J* 9.1 Hz, Ar-*H*), 6.78 (d, 2H, *J* 9.1 Hz, Ar-*H*), 5.87 (d, 1H, *J* 8.5 Hz, N*H*Ac), 5.46 (dd, 1H, $J_{1,2}$ 2.0 Hz, $J_{2,3}$ 3.4 Hz, H-2-Rha*p*), 5.33 (d, 1H, *J* 1.8 Hz, H-1-Rha*p*), 5.28 (t, 1H, $J_{2',3'}$, $J_{3',4'}$ 9.3 Hz, H-3-Glu*p*), 5.11 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.7 Hz, H-4-Rha*p*), 4.92 (d, 1H, *J* 8.2 Hz, H-1-Glu*p*), 4.67 (dd, 1H, *J* 4.5 Hz, 12.2 Hz, H-6-Glu*p*), 4.56 (dd, 1H, *J* 1.9 Hz, 12.2 Hz, H-6-Glu*p*), 4.27 (dd, 1H, $J_{2,3}$ 3.5 Hz, $J_{3,4}$ 9.7 Hz, H-3-Rha*p*), 3.90 (m, 1H, H-2-Glu*p*), 3.77 (s, 3H, $C_6H_4OCH_3$), 3.70–3.54 (m, 4H, 2× H-5, O*H*, H-4-Glu*p*), 2.12, 2.10, 2.08, 1.95 (4s, 12H, 4× CH_3CO), 1.16 (d, 3H, *J* 6.2 Hz, H-6-Rha*p*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.7, 170.4, 170.2, 169.8 (4× CH_3CO), 167.0 (C_6H_5CO), 155.1, 149.8, 117.7(2), 114.6(2) ($C_6H_4OCH_3$), 100.8, 96.3 (2× C-1), 75.0, 74.6, 74.0, 72.2, 71.4, 68.9, 66.7, 63.6, 55.5 ($C_6H_4OCH_3$), 55.1, 23.1, 20.9(2), 20.8, (4× CH_3CO), 17.3 (C-6-Rha*p*). ESI-MS *m*/*z* calcd for $C_{34}H_{41}NNaO_{15}$ $(M+Na)^+$ 726.2. Found: 726.3. Anal. Calcd for $C_{34}H_{41}NO_{15}$: C, 58.03; H, 5.87; N, 1.99. Found: C, 58.30; H, 5.65; N, 2.07.

4.2.12. *p*-Methoxyphenyl 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-mannopyranosyl-(1→2)-[2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-]4-*O*-benzoyl-α-L-rhamnopyranosyl-(1→4)-3-*O*-acetyl-4-*O*-benzoyl-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranoside (21)

Compound **21** (220 mg, 69%) was prepared by coupling of **20** (190 mg, 0.27 mmol) with **11** (390 mg, 0.33 mmol) under the same conditions as described for the synthesis of **7** by coupling of **4** with **6**. $[\alpha]_D^{25}$ + 50.3° (*c* 0.5 $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 8.07–8.03 (m, 4H, Bz-*H*), 7.91 (d, 2H, *J* 7.4 Hz, Bz-*H*), 7.81 (d, 2H, *J* 7.3 Hz, Bz-*H*), 7.69 (d, 2H, *J* 7.6 Hz, Bz-*H*), 7.55–7.27 (m, 14H, Bz-*H*), 7.19 (t, 2H, *J* 7.7 Hz, Bz-*H*), 6.91 (d, 2H, *J* 9.1 Hz, Ar-*H*), 6.78 (d, 2H, *J* 9.1 Hz, Ar-*H*), 5.90–5.85 (m, 2H, 2× N*H*Ac), 5.64 (dd, 1H, $J_{2,3}$ 3.0 Hz, $J_{3,4}$ 10.2 Hz, H-3-Rha*p*D), 5.60 (t, 1H, $J_{2,3}$, $J_{3,4}$ 9.4 Hz, H-

3-Glu*p*), 5.55–5.39 (m, 5H, H-2-Rha*p*A, H-2-Rha*p*D, H-3-Man*p*, H-4-Rha*p*C, H-4-Rha*p*D), 5.34 (d, 1H, *J* 1.6 Hz, H-1-Man*p*), 5.29 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.6 Hz, H-4-Man*p*), 5.21 (s, 1H, H-1-Rha*p*), 5.14 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.6 Hz, H-4-Rha*p*A), 5.08 (d, 1H, *J* 8.2 Hz, H-1-Glu*p*), 5.05 (s, 1H, H-1-Rha*p*), 4.92 (s, 1H, H-1-Rha*p*), 4.90–4.70 (m, 3H, H-2-Man*p*, H-2-Rha*p*C, H-3-Rha*p*C), 4.60–4.25 (m, 6H, H-3-Rha*p*A, 2× H-6-Man*p*, 3× H-5), 4.17 (m, 1H, H-2-Glu*p*), 4.10–3.80 (m, 3H, H-4-Glu*p*, H-5-Glu*p*, H-6-Glu*p*), 3.80–3.70 (m, 4H, H-6-Glu*p*, C_6H_4-OCH_3), 3.63 (m, 1H, H-5), 2.17–2.04 (m, 21H, 7× CH_3CO), 1.94 (s, 3H, CH_3CO), 1.37 (d, 3H, *J* 6.1 Hz, H-6-Rha*p*), 1.23 (d, 3H, *J* 5.7 Hz, H-6-Rha*p*), 1.16 (d, 3H, *J* 6.2 Hz, H-6-Rha*p*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.1, 170.3, 170.2(3), 170.1, 169.7(2) (8× CH_3CO), 166.1, 165.8, 165.2, 164.9, 164.5 (5× C_6H_5CO), 155.1, 149.8, 117.7(2), 114.5(2) ($C_6H_4OCH_3$), 100.8 (C-1-Glu*p*), 98.8, 98.3, 98.2 (3× C-1-Rha*p*), 96.2 (C-1-Man*p*), 75.2, 73.5, 73.2, 73.2, 72.7, 72.2, 72.1, 71.6, 71.3, 70.3, 69.3, 69.2, 69.0, 68.6, 67.7, 66.8, 65.0, 62.5, 62.1, 55.6, 55.5 ($C_6H_4OCH_3$), 50.7, 23.3, 23.1, 21.1, 20.9(2), 20.7(2), 20.6 8× CH_3CO), 17.7, 17.4, 17.2 (5× C-6-Rha*p*). ESI-MS *m*/*z* calcd for $C_{88}H_{96}KN_2O_{35}$ $(M+K)^+$ 1779.5. Found: 1779.8. HRMS for $C_{88}H_{97}N_2O_{235}$ $(M+H)^+$ 1741.5872. Found: 1741.5921.

4.2.13. *p*-Methoxyphenyl 2-acetamido-2-deoxy-α-D-mannopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)-]α-L-rhamnopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranoside (2)

Pentasaccharide **21** (0.16 g, 0.066 mmol) was dissolved in satd NH_3–MeOH (40 mL). After 1 week at rt, the reaction mixture was concentrated, and the residue was purified by chromatography on Sephadex LH-20 (MeOH) to afford **2** (79 mg, 89%) as a foamy solid. $[\alpha]_D^{25}$ −39.5° (*c* 0.3 $CHCl_3$). ^{1}H NMR (300 MHz, MeOD): δ 6.95 (d, 2H, *J* 8.4 Hz, Ar-*H*), 6.83 (d, 2H, *J* 8.2 Hz, Ar-*H*), 5.28 (s, 1H, H-1, H-1-Man*p*), 5.04 (s, 1H, H-1, H-1-Rha*p*), 4.97 (s, 1H, H-1, H-1-Rha*p*), 4.85 (s, 1H, H-1, H-1-Rha*p*), 4.61 (d, 1H, *J* 8.0 Hz, H-1-Glu*p*), 4.26–4.24 (m, 2H), 4.00–3.82 (m, 6H), 3.80–3.51 (m, 16H), 3.50–3.30 (m, 3H), 1.95, 1.94 (2s, 6H, 2× $COCH_3$), 1.25–1.15 (m, 6H, 2× H-6), 1.11 (d, 1H, *J* 5.9 Hz, H-6). ^{13}C NMR (75 MHz, MeOD): δ 174.8, 174.7 (2× $COCH_3$), 154.6, 149.3, 118.7(2), 115.1(2) (C_6H_4-OCH_3), 102.8 (C-1-Glu*p*), 101.7, 98.8, 97.2, 97.0 (4× C-1), 80.1, 77.5, 75.0, 74.8, 74.6, 72.8, 72.3, 72.1, 71.9, 70.7, 70.2, 69.9, 69.8, 69.7, 69.6, 69.0, 68.7, 66.4, 60.4, 60.2, 56.1, 55.8, 52.8 ($C_6H_4OCH_3$), 22.2, 21.9 (2× $COCH_3$), 16.7, 16.6, 16.4 (3× C-6). HRMS for $C_{41}H_{65}N_2O_{24}$ $(M+H)^+$ 969.3927. Found: 969.3909.

4.3. Biological activities

The plant growth regulation activity of the synthetic pentasaccharide was evaluated by a wheat coleoptile test, according to the procedures described previously.[21] Briefly, ten 4.0 mm wheat coleoptile sections were used in each Petri dish. Dilution series of both calibration indole-3-acetic acid (IAA) and the synthetic pentasaccharide were prepared with 0.01 M pH 5.0 phosphoric acid buffers. Length was measured after 24 h in the dark at 25 °C. Each treatment was replicated 4 times, and the experiment was carried out as a complete randomized block design.

To characterize the regulation effect, promotion rate (PR) is defined by either the synthetic pentasaccharide or IAA to the control (tap water) as

$$PR(\%) = (L_{treated} - 4.0)/(L_{control} - 4.0) \times 100$$

where *L* is the average length of coleoptile section.

4.4. Field experiment

The experiment was conducted in the China Agricultural University Shangzhuang Experiment Station (Haidian, Beijing) in 2011, using *Triticum aestivum* L. (cv. 'Jingdong 8'). The wheat seeds were immersed with 20 mg/L pentasaccharide aqueous solution for 8 h, and tap water as control. The soaked seeds were grown in the fertile loam soil on September 28. Before the wintering stage (November 15), the expansion leaves number of the main stem, till number, till number with no less than 3 leaves, and total root number of each plant were investigated. Each treatment was replicated 4 times, and the experiment was carried out as a completely randomized block design and each treatment had 50 plants.

Acknowledgments

This work was supported by the National Basic Research Program of China (Nos. 2011AA10A206, 2010CB126105, 2011BAE06 B02-01 and 2012BAK25B03-01), the NSFC (20902108 and 21172257) of China, and the Chinese Universities Scientific Fund (2011JS030).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2013.12.006.

References

1. Robina, I.; Lopez-Barba, E.; Jimenez-Barbero, J.; Martin-Pastor, M.; Fuentes, J. *Tetrahedron: Asymmetry* **1997**, *8*, 1207–1224.
2. Maier, R. J.; Brill, W. J. *J. Bacteriol.* **1978**, *133*, 1295–1299.
3. Maier, R. J.; Brill, W. J. *J. Bacteriol.* **1976**, *127*, 763–769.
4. Stacey, G.; Paau, A. S.; Brill, W. J. *Plant Physiol.* **1980**, *66*, 609–614.
5. Long, S. R. *Plant Cell* **1996**, *8*, 1885–1898.
6. Fedonenko, Y. P.; Egorenkova, I. V.; Konnova, S. A.; Ignatov, V. V. *Microbiology (Moscow, Russian Federation) (Translation of Mikrobiologiya)* **2001**, *70*, 329–334.
7. Jofre, E.; Lagares, A.; Mori, G. *FEMS Microbiol. Lett.* **2004**, *231*, 267–275.
8. Brink, R. A.; Miller, J.; Carlson, R. W.; Noel, K. D. *J. Bacteriol.* **1990**, *172*, 548–555.
9. de Maagd, R. A.; Rao, A. S.; Mulders, I. H. M.; Goosen de Roo, L.; van Loosdrecht, M. C. M.; Wijffelman, C. A.; Lugtenberg, B. J. J. *J. Bacteriol.* **1989**, *171*, 1143–1150.
10. Wang, Y.; Hollingsworth, R. I. *Carbohydr. Res.* **1994**, *260*, 305–317.
11. Zhang, J. J.; Zong, G. H.; Liang, X. M.; Li, Y. Q.; Wang, D. Q.; Kong, F. Z. *Chin. Chem. Lett.* **2008**, *19*, 415–418.
12. Zhao, H. Q.; Jia, H. Q.; Duan, H. X.; Zhang, J. J.; Liang, X. M.; Wang, D. Q. *J. Carbohydr. Chem.* **2010**, *29*, 103–117.
13. Grundler, G.; Schmidt, R. R. *Liebigs Ann. Chem.* **1984**, *11*, 1826–1847.
14. Briner, K.; Vasella, A. *Helv. Chim. Acta* **1987**, *70*, 1341–1356.
15. Zhang, M. M.; Du, Y. G.; Kong, F. Z. *Carbohydr. Res.* **2001**, *330*, 319–324.
16. Popelova, A.; Kefurt, K.; Hlavackova, M.; Moravcova, J. *Carbohydr. Res.* **2005**, *340*, 161–166.
17. Zong, G. H.; Yan, S. Q.; Liang, X. M.; Zhang, J. J.; Wang, D. Q.; Kong, F. Z. *Chin. Chem. Lett.* **2009**, *20*, 127–130.
18. Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21–123.
19. Byramova, N. E.; Ovchinnikov, M. V.; Bakinovskii, L. V.; Kochetkov, N. K. *Carbohydr. Res.* **1983**, *124*, C8–C11.
20. Yudina, O. N.; Gening, M. L.; Tsvetkov, Y. E.; Grachev, A. A.; Pier, G. B.; Nifantiev, N. E. *Carbohydr. Res.* **2011**, *346*, 905–913.
21. Jacobs, W. P.; Falkenstein, K.; Hamilton, R. H. *Plant Physiol.* **1985**, *78*, 844–848.