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TOTAL SYNTHESIS OF NONASACCHARIDE REPEATING UNIT OF PLANT CELL WALL XYLOGLUCAN: AN ENDOGENOUS HORMONE WHICH REGULATES CELL GROWTH[1]

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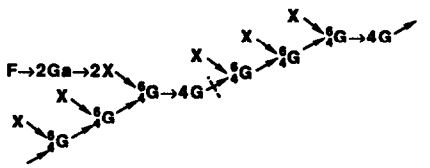
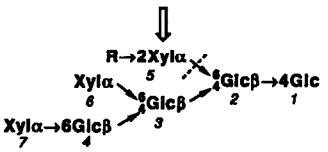
Abstract: Both glycoheptaosyl and glyconononaosyl repeating units of plant cell wall xyloglucan were synthesized for the first time in a stereocontrolled manner.

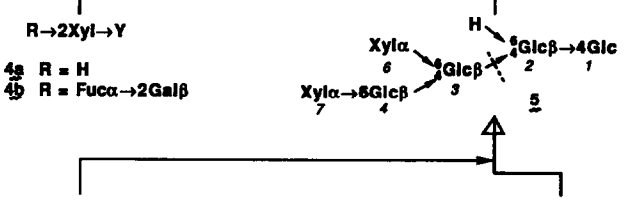
Xyloglucan **1** is present as a major component in the primary cell wall of dicots, monocots and gymnosperms, and contains equal amounts of two alternatingly repeating oligosaccharide fragments **2** and **3**, which have been isolated from cell walls and chemically characterized[2].



1 (X = α-D-Xyl, G = β-D-Glc, Ga = β-D-Gal, F = α-L-Fuc)



2 R = Fucα→2Galβ
3 R = H



4a R = H
4b R = Fucα→2Galβ

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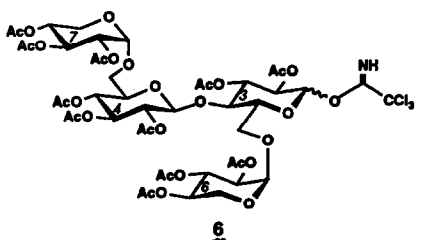

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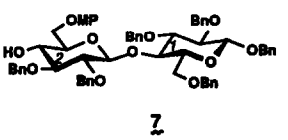

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Scheme 1 (MP = 4-MeOPh, Y = leaving group)

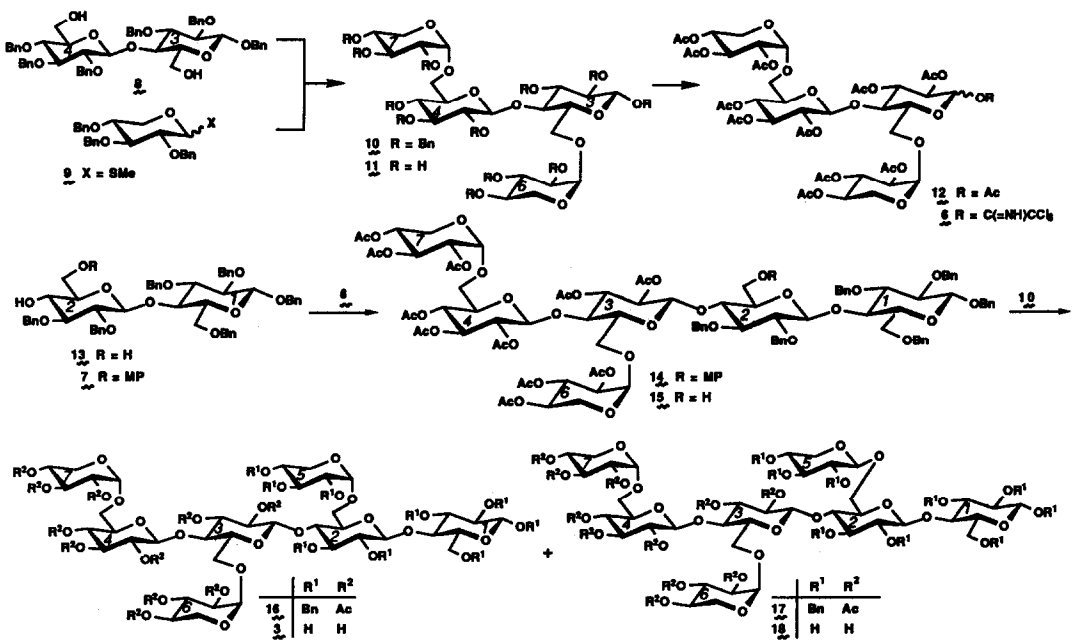
In 1984, York and co-workers reported[3] an inhibition of auxin stimulated growth of etiolated pea stem segments by oligosaccharide **2** but not **3**, both of which were isolated by digestion of suspension-cultured sycamore cell walls with endo-β-1,4-glucanase of *Trichoderma viride*. In 1988, this natural anti-auxin activity of **2** was confirmed by an independent bio-testing[4]. As part of our project on the synthetic studies directed toward plant cell wall-derived oligosaccharide fragments with biological functions[5], we now describe first total syntheses of **2** and **3** in a stereocontrolled manner.

Retrosynthetic analysis of **2** and **3** led us to design two glycosyl donors **4a** and **4b**, and a versatile glycohexaosyl acceptor **5** which in turn may be assembled from a properly protected

glycotetraosyl donor **6** and a glycobiosyl acceptor **7**. According to this scenario of a convergent approach to the synthesis of both **2** and **3**, a key intermediate **15**, a synthetic equivalent to **5**, was first synthesized as follows.

Stereocontrolled glycosylation of diol **8**[6], readily obtainable from β-D-Glc-(1→4)-β-D-Glc-(1→OBn[7] in 3 steps (*1* TrCl in Py, *2* BnBr, NaH in DMF, *3* 9:1 AcOH–H_2O, overall 50%), with methyl thioglycoside **9** in the presence of $CuBr_2$–Bu_4NBr–$HgBr_2$–powdered molecular sieves 4A (MS4A)[8] in CH_3NO_2 afforded **10** in 62% yield, the structure of which was confirmed by conversion into free glycotetraose **11**. Methyl thioglycoside **9** was conveniently prepared from corresponding trichloroacetimidate[9] by treatment with Bu_3SnSMe[10]. Conversion of **11** into glycosyl donor **6** was achieved in 3 steps via **12** (*1* Ac_2O in Py, *2* NH_2NH_2•AcOH in DMF[11], *3* CCl_3CN[12], DBU in CH_2Cl_2, overall 72%). Mitsunobu reaction[13] of diol **13**[14] with 4-MeOPhOH, $(EtOCON)_2$ and Ph_3P in $(CH_2Cl)_2$ afforded glycobiosyl acceptor **7** in 75% yield.

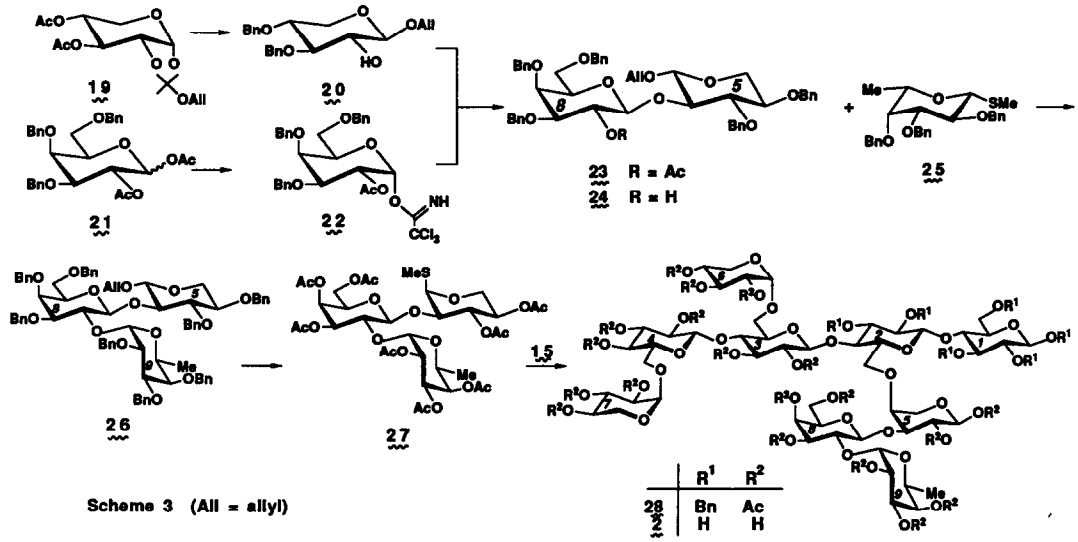
Coupling between glycobiosyl acceptor **7** and trichloroacetimidate **6** was performed in the presence of BF_3•OEt_2 to give a 53% yield of glycohexaoside **14**, which was converted in 56% yield into the key glycosyl acceptor **15** by treatment with CAN[15] in 4:1 CH_3CN–H_2O. Glycosylation of **15** with **9** in the presence of $CuBr_2$–Bu_4NBr–$HgBr_2$–MS4A in CH_3NO_2 proceeded with low stereoselectivity and gave in 64% yield a mixture of α-(1→6) linked product **16** and the β isomer **17** in a ratio of 4:3, which were separated and deblocked in 2 steps (*1* 0.1M NaOMe in MeOH, *2* Pd–C, H_2 in MeOH) into free glycoheptaose **3** (63%) and **18** (94%).



Scheme 2

Having prepared a glycoheptaosyl repeating unit **3** of **1**, development of a synthetic route to **2** was now examined by use of the key intermediate **15**. Methylthioglycoside **27** was designed

to play a role as a synthetic equivalent to the key glycotriosyl donor **4b** (scheme 1) and synthesized in a following way. Allyl xylopyranoside **20** was prepared from **19**[16] in 4 steps (*1* NaOMe in MeOH, *2* BnBr, NaH in DMF, *3* TMSOTf–MS4A[17] in $(CH_2Cl)_2$, *4* NaOMe in MeOH, overall 75%). Trichloroacetimidate **22** was obtained from diacetate **21**[18] in 3 steps (*1* $NH_2NH_2 \cdot H_2O$ in DMF, *2* CCl_3CN, DBU in CH_2Cl_2, overall 80%). $BF_3 \cdot OEt_2$–MS4A Promoted glycosylation of **20** with **22** afforded **23** (73%), which was saponified by NaOMe in MeOH to give **24** (94%). MeOTf–MS4A[19] Promoted glycosylation of **24** with **25**[20] afforded an 81% yield of α–(1→2) linked glycotrioside **26** along with a 7% yield of β–(1→2) linked isomer. Conversion of **26** into **27** was carried out in 7 steps (*1* $(Ph_3P)_3RhCl$, DABCO in 7:3:1 EtOH–PhH–H_2O[21], *2* $HgCl_2$–HgO in 9:1 Me_2CO–H_2O, *3* 10% Pd–C in 1:1 THF–MeOH, *4* Ac_2O in Py, *5* $NH_2NH_2 \cdot AcOH$ in DMF, *6* CCl_3CN, DBU in CH_2Cl_2, *7* Bu_3SnSMe, $BF_3 \cdot OEt_2$, MS4A in $(CH_2Cl)_2$, overall 31%). Crucial coupling between **15** and **27** proceeded in the



Scheme 3 (All = allyl)

presence of $CuBr_2$–Bu_4NBr–$HgBr_2$–MS4A in Et_2O to give a 26% yield of a mixture of α–(1→6) linked **28** and the β–(1→6) linked isomer in a ratio of 3:1. Deprotection of **28** afforded the target **2** in 2 steps (*1* NaOMe in MeOH, *2* 10% Pd–C in 2:1 MeOH–H_2O, then Sephadex G–25 in H_2O, overall 54%). ^1H–N.m.r. data of synthetic **2** and **3** were in good agreement with those[22] of natural samples, thus providing synthetic support for the proposed structures of **2** and **3** as the alternatingly repeating unit of plant cell wall xyloglucans.

In summary, unambiguous routes to the syntheses of target molecules **2** and **3** were developed for the first time by employing the alcohol **15** as a key glycohexaosyl acceptor.

Acknowledgment. We thank Prof. Y. Kato for kindly sending us natural samples of **2** and **3**. We thank Dr. J. Uzawa and Mrs. T. Chijimatsu for recording and measuring the n.m.r. spectra and Mrs. M. Yoshida and her staff for the elemental analyses. We also thank Ms. A. Takahashi and Ms. K. Moriwaki for their technical assistance.

Reference and Notes

1 Part 8 in the series "Synthetic studies on plant cell wall glycans". For part 7, see Y. Nakahara and T. Ogawa, *Carbohydr. Res.*, in press.

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6 Physical data for key compounds are described below. Values of $[\alpha]_D$ and $\delta_{H,C}$ were measured for $CHCl_3$ and $CDCl_3$ solution, respectively, at 23°±3°, unless noted otherwise. **2**: δ_H (D_2O, 60°) 5.275 (d, 3.7 Hz, 1^9), 5.220 (d, 3.7 Hz, $1^1\alpha$), 5.118 (d, 4.0 Hz, 1^5), 4.940 and 4.929 (2d, 4.0 Hz, $1^{6,7}$), 4.658 (d, 7.9 Hz, $1^1\beta$), 4.621 (d, 7.6 Hz, 1^8), 4.535 (d, 8.2 Hz, $1^{2,3,4}$), 1.257 (d, 6.7 Hz, 6^9). **3**: δ_H (D_2O, 60°) 5.219 (d, 4.0 Hz, $1^1\alpha$), 4.943 and 4.928 (2d, in a ratio of 2:1, 3.6 Hz, $1^{5,6,7}$), 4.654 (d, 8.3 Hz, $1^1\beta$), 4.562, 4.557 and 4.537 (3d, 8.0 Hz, $1^{2,3,4}$). **6**: $[\alpha]_D$ +95.9° (c 1.2); δ_H 8.651 (s, C=NH), 6.443 (d, 3.7 Hz, 1^3). **7**: $[\alpha]_D$ -15.6° (c 1.0); δ_H 4.526 and 4.485 (2d, 7.5 Hz, $1^{1,2}$), 3.760 (OMe). **8**: $[\alpha]_D$ -38.3° (c 1.0); δ_H 4.544 and 4.526 (2d, 7.8 Hz, $1^{3,4}$). **9**: (α:β=5:6); δ_H 5.193 (d, 5.2 Hz, 1α), 4.319 (d, 9.4 Hz, 1β), 2.201 (s, SMeβ), 2.032 (s, SMeα). **10**: $[\alpha]_D$ +41.0° (c 1.7); δ_C 102.6 and 102.5 ($1^{3,4}$), 97.2 and 97.0 ($1^{6,7}$). **11**: $[\alpha]_D$ +101.5° (c 1.0, H_2O); δ_H (D_2O) 5.226 (d, 3.7 Hz, $1^3\alpha$), 4.940 (d, 3.7 Hz, $1^{6,7}$), 4.688 (d, 8.2 Hz, $1^3\beta$), 4.559 (d, 7.9 Hz, 1^4). **12**: (α:β=7:9); δ_H 6.199 (d, 3.7 Hz, $1^3\alpha$), 5.610 (d, 8.3Hz, $1^3\beta$). **13**: $[\alpha]_D$ -6.3° (c 1.0). **14**: $[\alpha]_D$ +38.1° (c 1.3); δ_H 3.764 (s, OMe); δ_C 102.5, 102.1, 100.6 and 100.3 ($1^{1,2,3,4}$), 96.9 and 96.0 ($1^{6,7}$). **15**: $[\alpha]_D$ +36.2° (c 1.0); δ_H 2.106, 2.057, 2.029, 2.023, 2.011, 2.007 (x2), 1.987 (x2), 1.960 and 1.924 (9s, 11Ac). **16**: R_F 0.36 in 10:1 $CHCl_3$-Me_2CO; δ_H 2.107, 2.059, 2.048, 2.010, 2.003 (x2), 1.999 (x2), 1.984, 1.939, 1.780 (9s, 11Ac). **17**: R_F 0.33 in 10:1 $CHCl_3$-Me_2CO; δ_H 2.115, 2.069, 2.062, 2.048, 2.034, 2.015, 2.009, 2.007, 1.995, 1.962, 1.916 (11s, 11Ac). **18**: δ_H (D_2O, 60°) 5.199 (d, 4.0 Hz, $1^1\alpha$), 4.923 and 4.911 (2d, 3.7 Hz, $1^{6,7}$), 4.631 (d, 8.1 Hz, $1^1\beta$), 4.567, 4.520, 4.512 and 4.441 (4d, 8.0 Hz, $1^{2,3,4,5}$). **19**: m.p. 44-46° (EtOAc-hexane); δ_H 5.571 (d, 4.4 Hz, 1), 2.115 and 2.091 (2s, 2Ac), 1.748 (s, C-Me). **20**: $[\alpha]_D$ -36.8° (c 1.0). **22**: δ_H 8.502 (s, C=NH), 6.529 (d, 3.6 Hz, 1). **23**: δ_H 5.390 (dd, 8.2 and 10.1 Hz, 2^8), 4.778 (d, 8.2 Hz, 1^8), 1.772 (s, Ac). **26**: $[\alpha]_D$ -51.0° (c 1.0); m.p. 107.5-108.5° (EtOAc-hexane); δ_H 5.760 (d, 3.7 Hz, 1^9), 4.868 (d, 7.6 Hz, 1^8), 4.314 (d, 6.7 Hz, 1^5), 1.138 (d, 6.4 Hz, 6^9); δ_C 101.5 and 100.6 ($1^{5,8}$), 97.5 (1^9). **27**: $[\alpha]_D$ -79.3° (c 1.0); δ_H 5.332 (d, 4.3 Hz, 1^9), 5.257 (d, 5.5 Hz, 1^5), 4.558 (d, 7.6 Hz, 1^8). **28**: $[\alpha]_D$ +18.3° (c 0.9); δ_H 2.214, 2.125, 2.115, 2.064, 2.053, 2.048, 2.014, 2.010, 2.008 (x2), 2.003, 1.997, 1.981, 1.976, 1.963, 1.956, 1.954, 1.928 and 1.921 (18s, 19Ac), 1.203 (d, 6.6 Hz, 6^9).

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16 **19** was prepared from 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide by treatment with CH_2=$CHCH_2OH$, Bu_4NBr, Et_3N in CH_2Cl_2 (95%).

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