Carbohydrate Research, 243 (1993) C1–C7
Elsevier Science Publishers B.V., Amsterdam

Preliminary communication

Stereocontrolled synthesis of chitosan dodecamer †

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(Received December 28th, 1992; accepted with revision February 1st, 1993)

Along with β-glucan, chitin and chitosan are major structural components of fungal cell walls. In the incompatible interactions between pathogenic fungi and plants, fragments of these polysaccharides play significant roles in eliciting defense-related responses in various plants[2]. Chitosan applied to pea endocarp tissue activated the disease resistance responses as did inoculation with incompatible pathogens[3]. The effective glucosamine oligomer size was proposed to be a heptamer or larger[4]. Similar chitosan-induced defense-related responses were observed in other plants such as soybean[5] and parsley[6]. Chitosan-derived oligosaccharides were also powerful inducers of proteinase inhibitors in excised tomato leaves[7]. On the other hand, chitin oligomers, but not chitosan oligomers, elicit lignification in wounded wheat leaves[8]. In order to investigate the structural requirements for bioactive chitosan- and chitin-derived oligosaccharides in detail, we have carried out synthetic studies and describe here a stereocontrolled synthesis of chitosan oligomers up to a dodecamer **1** ($n = 5$) with a 4-methoxyphenyl group at the reducing end.

Synthesis of the target molecules **1** ($n = 1$–5) was carried out by employing trichloracetimidates **2** as key glycosyl donors and *O*-benzyl-*N*-phthaloyl derivatives **3** ($n = 1$–3) as key glycosyl acceptors, which were in turn prepared starting from a glycobiosyl donor **4** and a glycobiosyl acceptor **5**. Compounds **4** and **5** were prepared from readily available monosaccharide derivatives **6** (ref 9) and **7** (ref 10). Glycosylation of compound **6** with trichloroacetimidate **7** in the presence of $BF_3 \cdot OEt_2$ in CH_2Cl_2 at −78°C gave 83% of disaccharide **8**: R_f 0.55 in 3:1 (PhMe)–EtOAc; $[\alpha]_D$ +58.0° (*c* 2.0); δ_H (anomeric H) 5.448 and 5.334 (2 d, 8.6 Hz). (It should be noted that all new compounds described herein gave satisfactory data for the elemental analyses.) Optical rotations were determined for solutions

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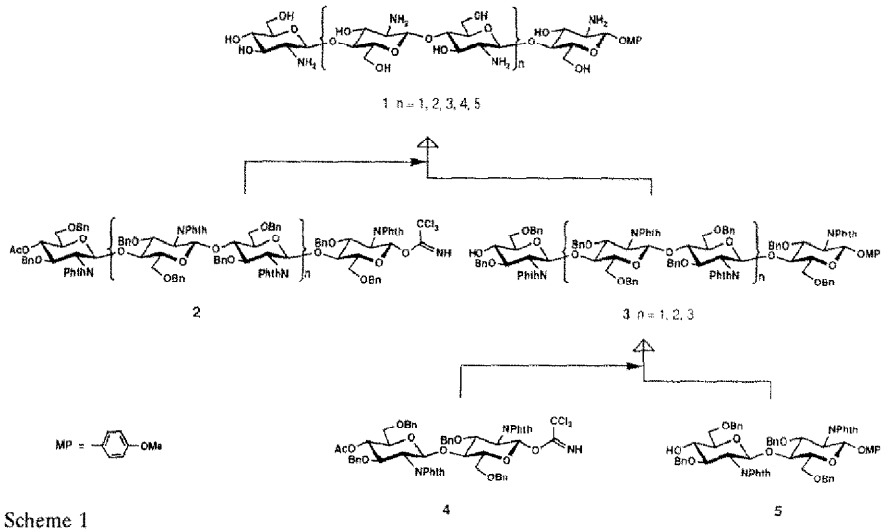
† Part 10 in the series, "Synthetic studies on plant cell wall glycans". For Part 9, see ref 1.

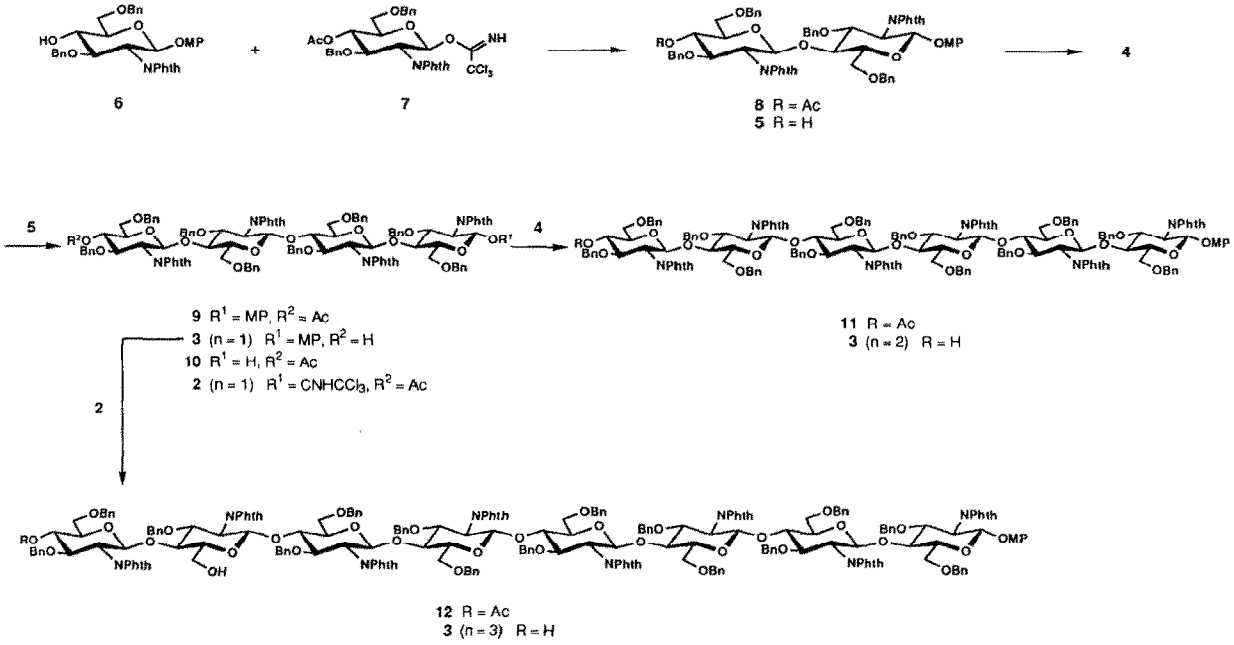


Scheme 1

in $CHCl_3$ at 25 ± 3°C. NMR spectra were recorded with either Jeol JNM-GX 400, JNM-GSX 500, or JNM-A600 spectrometers. The values of δ_H are expressed in ppm downfield from the signal for internal Me_4Si for solutions in $CDCl_3$ at 25°C, unless noted otherwise.

Oxidative removal of the methoxyphenyl group of **8** with $(NH_4)_2Ce(NO_3)_6$ (CAN) in 4:3:3 CH_3CN–H_2O–PhMe[11] and subsequent treatment of the product hemiacetal with CCl_3CN[12] and DBU in $(CH_2Cl)_2$ at 0°C gave 82% of the glycobiosyl donor **4**: R_f 0.53 in 1:1 hexane–EtOAc; $[\alpha]_D$ +49.1° (*c* 1.7); δ_H (anomeric H) 6.230 (d, 8.9 Hz), and 5.350 (d, 8.2 Hz). On the other hand, saponification of compound **8** was carried out in 94% yield by 0.01 M NaOMe in 4:1 MeOH–THF to give alcohol **5**: R_f 0.73 in 1:1 hexane–EtOAc; $[\alpha]_D$ +32.0° (*c* 2.8); δ_H (anomeric H) 5.446 (d, 8.6 Hz) and 5.313 (d, 8.2 Hz). Glycosylation of compound **5** with trichloroacetimidate **4** (1.5 equiv) was promoted in CH_2Cl_2 in the presence of $BF_3 \cdot OEt_2$–powdered AW300 molecular sieves at −78°C to give 75% of tetrasaccharide **9**: R_f 0.28 in 5:2 hexane–EtOAc; $[\alpha]_D$ +41.1° (*c* 1.6); δ_H (anomeric H) 5.374 (d, 8.5 Hz), 5.295 (d, 8.2 Hz), 5.048 (deformed d), and 5.078 (d, 7.9 Hz). A 99% conversion of compound **9** into a glycosyl acceptor **3** ($n = 1$) was achieved in by 1:2:15 1 M LiOH–30% aq H_2O_2–THF[13] at 0°C. Compound **3** ($n = 1$) had R_f 0.30 in 5:1 PhMe–Me_2CO; $[\alpha]_D$ +24.3° (*c* 1.7); δ_H 5.372 (d, 8.5 Hz), 5.261 (d, 8.2 Hz), 5.076 (d, 7.9 Hz), and 5.044 (deformed d).

Compound **9** was converted as described for compound **4** in two steps in 84.5% overall yield into compound **2** ($n = 1$): R_f 0.71 in 10:3 PhMe–Me_2CO; $[\alpha]_D$ +40.3° (*c* 0.9); δ_H (anomeric H) 6.152 (d, 8.9 Hz), 5.292 (d, 8.2 Hz), 5.085 (d, 8.2 Hz), and 5.045 (d, 7.9 Hz). $BF_3 \cdot OEt_2$-promoted glycosylation of glycosyl acceptor **3** ($n = 1$) with glycosyl donor **4** (3 equiv) in 1:1 CH_2Cl_2–PhMe at −78°C afforded 62% of hexasaccharide **11**: R_f 0.16 in 1:1 hexane–EtOAc; $[\alpha]_D$ +28.9° (*c* 0.8); δ_H


6
7
8 R = Ac
5 R = H
4
5
9 R¹ = MP, R² = Ac
3 (n = 1) R¹ = MP, R² = H
10 R¹ = H, R² = Ac
2 (n = 1) R¹ = CNHCCl3, R² = Ac
11 R = Ac
3 (n = 2) R = H
2
12 R = Ac
3 (n = 3) R = H

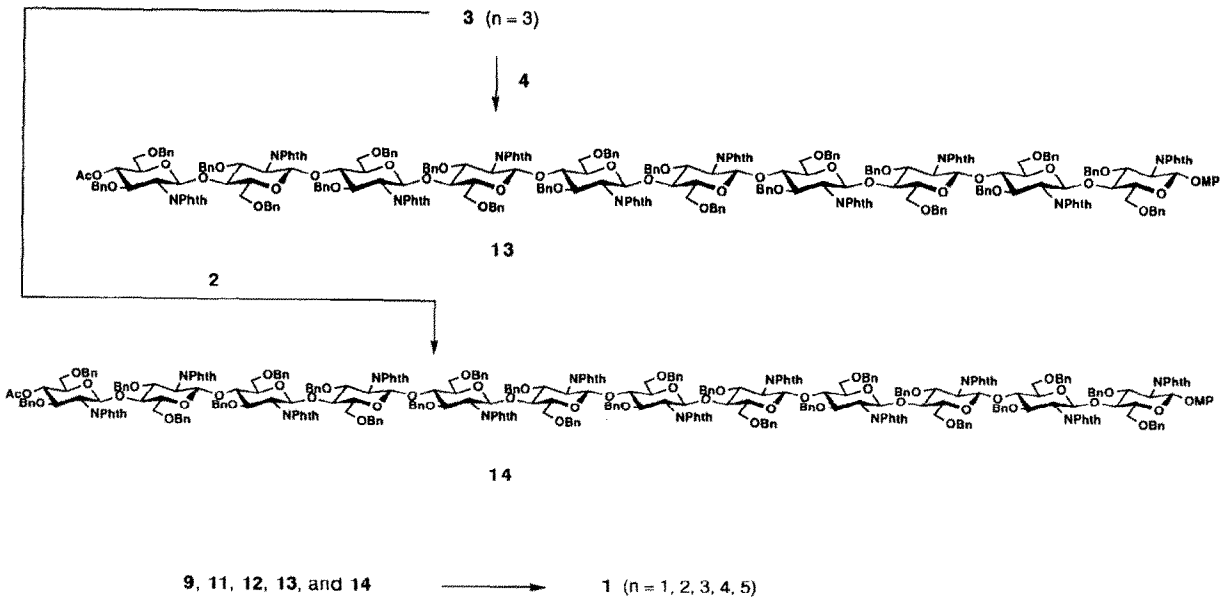
Scheme 2

TABLE I

Physical data for the synthetic chitosan oligomers [a]

1	$[\alpha]_D$	FABMS $(M+H)^+$	R_f in 1:2 aq NH_4OH(3%) -MeOH	^1H NMR (H-1) at 60°C	^{13}C NMR (C-1) at 20°C
$n=1$	−21.7° (*c* 0.5)	769.0	0.78	5.308 (8.2), 4.900 (8.5), 4.877 (8.2), 4.851 (8.2)	98.79, 98.74, 98.67, 98.42
$n=2$	−28.4° (*c* 0.3)	1091.3	0.53	5.312 (8.6), 4.918 (8.5), 4.897 (8.6), 4.891 (8.3), 4.889 (8.3), 4.860 (8.3)	98.57, 98.34×4, 98.24
$n=3$	−15.7° (*c* 1.0)	1413.6	0.56	5.301 (8.4), 4.891 (8.4), 4.858 (8.1)×5, 4.843 (8.4)	98.73, 98.64×6, 98.38
$n=4$	−13.0° (*c* 1.5)	1735.6	[b]	5.313 (8.2), 4.919 (8.2), 4.893 (8.2)×7, 4.859 (8.2)	98.36×10
$n=5$	−1.50° (*c* 0.5)	[c]	[b]	5.329 (7.9), 4.887 (7.9)×11	98.97×12

[a] The values of $[\alpha]_D$ were determined for solutions in H_2O at 25±3°C. The values of $\delta(D_2O)$ are expressed in ppm downfield from the signal for Me_4Si by reference to internal Me_3COH (1.230 ppm for ^1H 30.455 ppm for ^{13}C). Values in parentheses are $^3J_{HH}$ (Hz). [b] In the solvent systems so far examined, the original spot did not move. [c] Molecular ion could not be detected in various matrixes.



Scheme 3

(anomeric H) 5.354 (d, 8.8 Hz), 5.273 (d, 8.4 Hz), 5.036 (d, 8.1 Hz), and 5.001 (3 d, br). On the other hand, coupling between compound **3** ($n = 1$) and glycosyl donor **2** ($n = 1$, 0.5 equiv) under the same conditions as for compound **11** afforded a 64% yield of octasaccharide **12**: R_f 0.30 in 2:1 CCl_4–EtOAc; $[\alpha]_D$ +30.1° (*c* 1.1); δ_H (anomeric H) 5.349 (d, 8.5 Hz), 5.268 (d, 8.2 Hz), 5.109 (d, 9.2 Hz), 5.026 (d, 8.2 Hz), and 4.964 (br signals for 4 H). Compound **12** was converted with 88% yield of the corresponding alcohol **3** ($n = 3$) as described for compound **3** ($n = 1$). Compound **3** ($n = 3$) had R_f 0.28 in 2:1 CCl_4–EtOAc; $[\alpha]_D$ +22.6° (*c* 0.5); δ_H (anomeric H) 5.348 (d, 8.6 Hz), 5.238 (d, 8.2 Hz), 5.028 (d, 7.6 Hz), and 4.965 (br signals, 5 H). Glycosylation of alcohol **3** ($n = 3$) with glycosyl donor **4** (2.5 equiv) as for compound **12** gave a 65% yield of decasaccharide **13**: R_f 0.25 in 12:1 $CHCl_3$–EtOAc; $[\alpha]_D$ +26.8° (*c* 0.8); δ_H 5.346 (d, 8.5 Hz), 5.265 (d, 8.2 Hz), 5.109 (d, 9.2 Hz) for 3 anomeric H, 4.943 (br signals, 7 anomeric H), 3.608 (s, OMe), and 1.867 (s, OAc). Similarly glycosylation of alcohol **3** ($n = 3$) with glycosyl donor **2** ($n = 1$, 3 equiv) afforded a 49% yield of dodecasaccharide **14**: R_f 0.36 in 30:1 $CHCl_3$–THF; $[\alpha]_D$ +16.9° (*c* 1.3); δ_H 5.345 (d, 8.5 Hz), 5.263 (d, 8.3 Hz), 5.018 (d, 7.6 Hz) for 3 anomeric H, 4.930 (br signals for 9 anomeric H), 3.609 (s, OMe), and 1.868 (s, OAc).

Having prepared completely protected chitosan oligomers **9**, **11**, **12**, **13**, and **14**, they were respectively converted into chitosan oligomers **1** ($n = 1$–5; see Table I) in 60–85% yields in two steps: *1*, $NH_2NH_2 \cdot H_2O$ in refluxing MeOH; *2*, H_2, Pd-black in 1:1 0.1 M HCl–MeOH.

In summary, chitosan oligomers of plant physiological interest have been prepared up to the dodecamer in a stereo-controlled manner.

ACKNOWLEDGMENTS

A part of this work was financially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, and also by the Special Coordination Funds of the Science and Technology Agency of the Japanese Government. We thank Dr. J. Uzawa and Mrs. T. Chijimatsu for recording the NMR data, Mr. Y. Esumi for the FABMS data, Ms. M. Yoshida and her staff for elemental analyses, and Ms. A. Takahashi for technical assistance.

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