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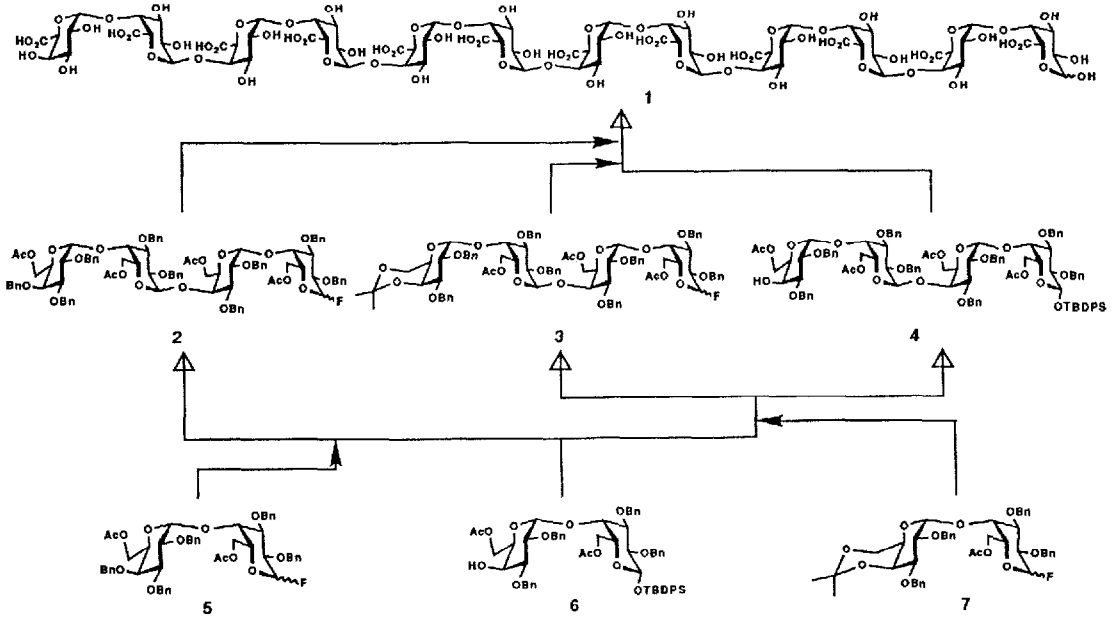
TOTAL SYNTHESIS OF GALACTODODECAOSIDURONIC ACID, AN ENDOGENOUS PHYTOALEXIN ELICITOR ISOLATED FROM SOYBEAN CELL WALL[1)]

Yoshiaki Nakahara* and Tomoya Ogawa*
RIKEN (The Institute of Physical and Chemical Research)
Wako-shi, Saitama, 351-01 Japan

Abstract: A first total synthesis of endogenous phytoalexin elicitor-active galactododecaosiduronic acid was achieved based on a highly stereocontrolled glycan chain elongation strategy employing three key galactotetraosyl intermediates. The suitably protected galactododecaoside was successfully transformed into the target galactododecaosiduronic acid.

Recent plantphysiological studies[2)] on galactooligosiduronic acids isolated from plant cell-wall pectin either by partial acid hydrolysis or by enzymic degradation clearly demonstrated a new defense mechanism against invading pathogens. Based on the extensive studies on phytoalexin accumulation induced by the fragments of plant cell-wall pectins, in 1983 galactododecaosiduronic acid **1** was proposed as a most active endogenous elicitor[3)].

As part of a project on the synthesis of plant cell-wall glycans of biological significance, particularly in association with their roles in self-defense mechanisms of plant cells, in 1987 we established a highly stereoselective strategy for the glycan chain extension[4)] toward α-(1→4)-



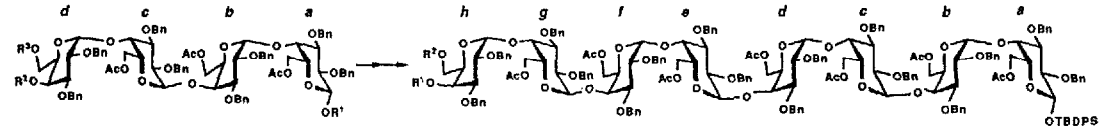
Scheme 1 (TBDPS = $tBuPh_2Si$)

linked galactooligosides along with their oxidative conversion[1] into galactooligosiduronic acids. We now describe an unambiguous synthesis of galactododecaosiduronic acid **1**.

Based on the retrosynthetic analysis aimed at a convergent synthesis of target **1**, three key galactotetraosyl intermediates **2**, **3**, and **4** were designed. According to our previous experiments[1], each glycotetraosyl intermediate may in turn be prepared stereoselectively by use of a galactobiosyl acceptor **6**[5] and either a galactobiosyl donor **5**[4] or **7**[4].

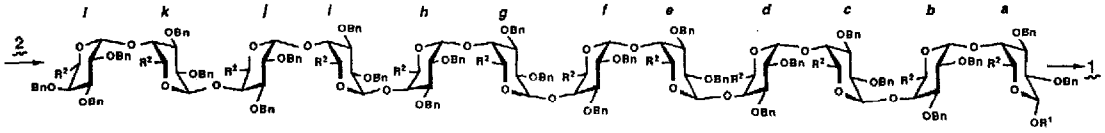
Glycosylation of **6** with **5** was promoted with $SnCl_2$-$AgClO_4$[6] in Et_2O to give a 92% yield of α-(1→4)-linked product **8**[7] as a sole product. Configuration at C-1*c*, the newly formed anomeric center of **8**, was assignable as α-D from ^{13}C nmr data[8]. Desilylation of **8** with n-Bu_4NF-AcOH[9] in THF gave a 90% yield of **9**, which was further treated with DAST[10] in THF to afford the designed galactotetraosyl donor **2**[7] in 97% as an anomeric mixture of α:β=7:18. Similarly, galactotetraosyl unit **3** corresponding to the middle part of **1** was efficiently prepared in 3 steps starting from **6** and **7**. A $SnCl_2$-$AgClO_4$ promoted coupling of **6** and **7** in Et_2O afforded a 77% yield of **10**[7] which was further converted in 2 steps as described above into **3**[7] as an anomeric mixture of α:β=8:17 in 91% overall yield. Compound **10** was also converted in 2 steps via **12**[7] into galactotetraosyl acceptor **4**[7] corresponding to the reducing end part of **1** in 86% overall yield (1 80% aq.AcOH at 60°, 2 AcCl in pyridine at -5°).

The key glycosylation between the two galactotetraosides **3** and **4** was again performed successfully in the presence of $SnCl_2$ and $AgClO_4$ in Et_2O and the expected product **13** was deisopropylidenated with 80% aq.AcOH to give galactooctaoside **14**[7] in 62% overall yield[11]. Selective acetylation (AcCl-pyridine at -5°) of **14** afforded an 82% yield of galactooctaosyl acceptor **15**[7], which in turn was glycosylated with **2** (1.5 eq.) in the presence of $SnCl_2$-$AgClO_4$ in 7:3 Et_2O-toluene at -10°~room temp. overnight to give exclusively galactododecaoside **16**[7] in 64% yield.



	R^1	R^2	R^3
8	TBDPS	Bn	Ac
9	H	Bn	Ac
10	TBDPS	$C(CH_3)_2$	
11	H	$C(CH_3)_2$	
12	TBDPS	H	H

	R^1	R^2
13	$C(CH_3)_2$	
14	H	H
15	H	Ac



	R^1	R^2
16	TBDPS	CH_2OAc
17	〃	CH_2OH
18	〃	CHO
19	〃	COOH
20	〃	CO_2CH_3
21	H	COOH

Scheme 2

With the desired chain length of galactododecaoside **16** prepared, further oxidative transformation into uronic acid **1** was examined as follows. Deacetylation with NaOMe-MeOH afforded an 81% yield of **17** which was oxidized in 2 steps to give crude **19** in 50% overall yield (1 DMSO-$(COCl)_2$-i-Pr_2EtN[12], 2 $NaClO_2$-2-methyl-2-butene[13]). In order to perform further purification[14], **19** was treated with CH_2N_2 in Et_2O to afford the corresponding dodecacarboxylic acid methyl ester **20**[7] in 80% yield after separation by preparative tlc. Regenaration of **19**[7] was achieved in 67% yield by refluxing a pyridine solution of **20** with excess LiI[15] for 30 h and then preparative tlc in 9:1:1 $CHCl_3$-Me_2CO-AcOH. Desilylation of **19** with n-Bu_4NF-AcOH in THF for 5 days at room temperature afforded a 75% yield of **21**, which was further hydrogenolysed with 10% Pd-C in 80% aq.MeOH for 4 days to afford a completely deblocked product. The product thus obtained was fractionated on an anion exchange resin (Mono Q, Pharmacia FPLC system[16]) with gradient elution by NH_4HCO_3 buffer to give a 75% yield of pure galactododecaosiduronic acid **1**. The ^{1}H-nmr data of **1** was in agreement with those[17] of the related natural products.

In summary, a first total synthesis of galactododecaosiduronic acid **1**, most active endogenous phytoalexin elicitor, was achieved under high stereocontrol.

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7) Physical data for new compounds are given below. Values of $[\alpha]_D$ and $\delta_{H,C}$ were recorded for solutions in $CHCl_3$ and $CDCl_3$, respectively, at 25° unless noted otherwise. **8**: Rf 0.67 (7:3 toluene-EtOAc); $[\alpha]_D$ +47.6° (c 0.8); δ_H 1.12 (s, tBu), 1.78, 1.89, 1.90, and 1.93 (4 s, 4 Ac); δ_C 98.0 (161 Hz, 1*a*), 99.4 (167 Hz) and 99.7 (169 Hz in a ratio of 2:1 for 1*bcd*); **2**: Rf 0.51 and 0.46 (7:3 toluene-EtOAc); δ_H 1.78 (s, Ac), 1.89 and 1.90 (2s, Ac), 1.92 and 1.95 (2s, Ac), 2.04 (s, Ac), 5.18 (dd, 5.8, 52.8 Hz, 1*a*β), 5.58 (dd, 2.2, 56.2 Hz, 1*a*α); **10**: Rf 0.64 (7:3 toluene-EtOAc); $[\alpha]_D$ +64.3° (c 1.0); δ_H 1.13 (s, tBu), 1.33 and 1.41 (2 s, CMe_2), 1.89, 1.90 and 2.01 (3 s, 3 Ac); δ_C 98.0 (1*a*), 99.2, 99.4 and

100.2 (3 s, 1*bcd*); **3**: Rf 0.51 and 0.48 (7:3 toluene-EtOAc); δ_H 1.33 and 1.41 (2s, CMe_2), 1.92 and 1.95 (2s, Ac), 1.97 and 1.98 (2s, Ac), 5.18 (dd, 6.1, 52.8 Hz, 1aβ), 5.59 (dd, 2.1, 55.5 Hz, 1aα); **12**: Rf 0.48 (3:2 EtOAc-n-hexane); $[\alpha]_D$ +63.4° (c 1.0); δ_H 1.13 (s, t-Bu), 1.90 (s, 2 Ac), 1.95 (s, Ac), 4.55 (d, 7.0 Hz, 1*a*), 4.96 and 4.98 (2d, 3.4 Hz, 1*bcd*); δ_C 98.0 (159 Hz, 1*a*), 99.2, 99.4 and 99.8 (173 Hz, 1*bcd*); **4**: Rf 0.42 (7:3 toluene-EtOAc); $[\alpha]_D$ +57.3° (c 0.6); δ_H 1.12 (s, tBu), 1.89, 1.90, 1.91 and 1.94 (4 s, 4 Ac), 3.22 (t, 6.7 Hz, 5*a*), 3.23 (dd, 10.1, 2.7 Hz, 3*a*), 3.64 (dd, 10.1, 7.3 Hz, 2*a*), 4.55 (d, 7.3 Hz, 1*a*), 4.95 (m, 1*bcd*); δ_C 98.0 (1*a*), 99.3 and 99.6 (2:1, 1*bcd*); **14**: Rf 0.26 (7:3 toluene-EtOAc); $[\alpha]_D$ +61.9° (c 0.7); δ_H 1.11 (s, tBu), 1.88 (s, 3 Ac), 1.89 (s, Ac), 1.90 (3 s, 3 Ac), 3.21 (brt, 5*a*), 3.25 (dd, 9.8, 2.8 Hz, 3*a*), 3.38 and 3.44 (2m, 6*h*), 3.63 (dd, 9.8, 7.3 Hz, 2*a*), 4.53 (d, 7.3 Hz, 1*a*); δ_C 98.1 (160 Hz, 1*a*), 99.3 and 99.8 (6:1, 170 Hz, 1*bcdefgh*); **15**: Rf 0.41 (7:3 toluene-EtOAc); $[\alpha]_D$ +53.8° (c 0.7); δ_H 1.12 (s, tBu), 1.868, 1.879, 1.885, 1.896, 1.899, 1.901, 1.904, 1.954 (8 s, 8 Ac), 3.23 (brt, 5*a*), 3.25 (dd, 9.8, 2.8 Hz, 3*a*), 3.63 (dd, 9.8, 7.0 Hz, 2*a*), 4.54 (d, 7.0 Hz, 1*a*); **16**: Rf 0.60 (7:3 toluene-EtOAc); $[\alpha]_D$ +55.8° (c 0.6); δ_H 1.12 (s, tBu), 1.78, 1.85, 1.88, 1.89, 1.90, 1.91, 1.96 (1:1:1:6:1:1:1, 7s, 12 Ac), 3.21 (brt, 5*a*), 3.24 (dd, 9.8, 2.4 Hz, 3*a*), 3.63 (dd, 9.8, 7.0 Hz, 2*a*), 4.56 (d, 7.0 Hz, 1*a*); δ_C 97.9 (1*a*), 99.2, 99.3, 99.4 and 99.6 (8:1:1:1, 5s, 1*bcdefghijkl*); **17**: Rf 0.26 (3:2:5 toluene-$CHCl_3$-EtOAc); $[\alpha]_D$ +79.1° (c 0.5); δ_H 1.09 (s, t-Bu), 4.55 (d, 7.3 Hz, 1*a*); **20**: Rf 0.56 (7:3 toluene-EtOAc); δ_H 1.170 (s, tBu), 3.087, 3.194, 3.199 (6 H), 3.204 (6 H), 3.208, 3.237, 3.253, 3.365, 3.443 and 3.449 (10 s, 12 Me); **19**: $[\alpha]_D$ +100.3° (c 0.9); **1**: $[\alpha]_D$ +80.8° (c 0.1, H_2O); δ_H (D_2O, 80°, tBuOH) 3.49 (dd, 7.6, 10.1 Hz, 2aβ), 3.76 (bd, 2*bcdefghijkl* and 3*a*), 3.90 (dd, 3.7, 10.1 Hz, 3*l*), 3.98 (bd, 3*bcdefghijk*), 4.45 (bd, 4*bcdefghijkl*), 4.58 (d, 7.6 Hz, 1aβ), 4.77 (bs, 5*bcdefghijkl*), 5.10 (bs, 1*bcdefghijkl*), 5.31 (bd, 1aα).

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14) Purification at the stage of the ester **20** was necessary to remove the contaminating materials produced during the oxidation. Direct deprotection of the acid **19**, skipping this process, ended up with the formation of a mixture of the products from which **2** was isolable only with difficulty.

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