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Studies on the synthesis of two tetrasaccharides and the reactivity difference between them [1]

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Abstract

Studies on the reactivity of two synthetic tetrasaccharides as glycosyl acceptors showed that condensation of the methyl α-glycoside with a disaccharide donor afforded a hexasaccharide, but condensation of the methyl β-glycoside with the disaccharide did not yield the corresponding hexasaccharide under the same conditions. A combination of theoretical results and 2D NMR indicated that the reactivity difference between the methyl α-glycoside and the methyl β-glycoside was determined mainly by steric effects. © 1997 Elsevier Science Ltd.

Keywords: Tetrasaccharide; Reactivity; Conformation

1. Introduction

Laminin is an important glycoprotein in basement membrane. It can promote cell adhesion and migration, and is believed to play a role in tumor cell invasion [1]. Because of the role of laminin carbohydrates on cellular interactions [2], we tried to synthesize the core structure of the oligosaccharide of laminin and its analogues to explore the possible prevention of metastatic spread. Unexpectedly, synthesis of methyl (2,3,4,6 - tetra - *O* - acetyl - β - D - galactopyranosyl)-(1 → 4)-(3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 2)-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-(3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 6)]-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 6)-(2,3,4-tri-*O*-benzyl-β-D-mannopyranoside) (**12**) and methyl (2,3,4,6 - tetra - *O* - acetyl - β - D - galactopyranosyl)-(1 → 4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1 → 2)-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1 → 6)]-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 6)-(2,3,4-tri-*O*-benzyl-β-D-mannopyranoside) (**10**) was not successful. However synthesis of their α-anomers–methyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-(3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 2)-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-(3,6 - di - *O* - acetyl - 2 - deoxy - 2 - phthalimido - β - D -

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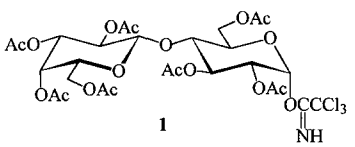
glucopyranosyl)-(1 → 6)]-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranoside) (**11**) and methyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1 → 2)-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1 → 6)]-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranoside) (**9**) was carried out readily [3]. In order to understand this phenomenon, two tetrasaccharides–methyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1 → 6)-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 6)-(2,3,4-tri-*O*-benzyl-β-D-mannopyranoside) (**7**) and methyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1 → 6)-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranoside) (**8**) were synthesized, and then their reactivity differences and conformations were studied.

2. Results and discussion

Synthesis of two of tetrasaccharides.—Tetrasaccharide **7** was synthesized from 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl) trichloroacetimidate (**1**) [4], 2,6-di-*O*-acetyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (**2**) and methyl 2,3,4-tri-*O*-benzyl-β-D-mannopyranoside (**3**) obtained as described in refs. [3,5]. Condensation of **2** with **3** in CH_2Cl_2 in the presence of Me_3SiOTf gave **4** in 90% yield. Compound **4** was *O*-deacetylated to afford **5** (95%). Coupling of **5** with **1** in CH_2Cl_2 using $Et_2O \cdot BF_3$ as a promoter gave **7** in 57% yield.



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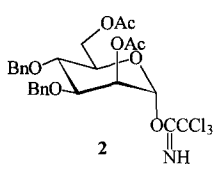

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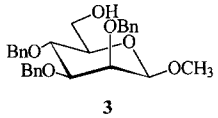

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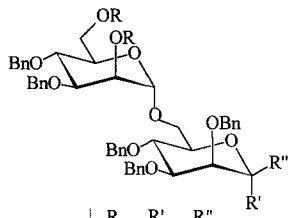
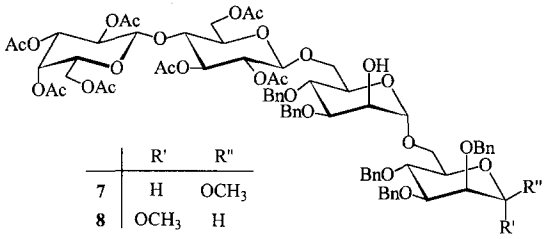


	R	R'	R''
4	Ac	H	OCH_3
5	H	H	OCH_3
6	H	OCH_3	H

For the synthesis of tetrasaccharide **8**, we employed 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl) trichloroacetimidate (**1**) as the glycosyl donor and methyl 3,4-di-*O*-benzyl-α-D-mannopyranosyl-(1 → 6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranoside) (**6**) [3] as the glycosyl acceptor. Condensation of **1** with **6** afforded **8** in 73% yield.



	R'	R''
7	H	OCH_3
8	OCH_3	H

Reactivity of tetrasaccharide **7** *and tetrasaccharide* **8**.—In order to investigate the glycosylation of disaccharide donor **1** and tetrasaccharide acceptors **7** and **8**, two coupling reactions were carried out separately in two flasks at the same time under exactly the same conditions as given in the Experimental for preparing compound **9**. In one flask, condensation of **1** with **7** was carried out in CH_2Cl_2 using $Et_2O \cdot BF_3$ as activator. After 12 h, the mixture was processed conventionally. The condensation product was not obtained, and instead the donor and the acceptor were recovered. In another flask, coupling of **1** with **8** gave the desired condensation product **9** in 65% yield. Under different conditions (Table 1), coupling of **1**

Table 1

Acceptor	Donor	Catalyst	Temperature	Time	Hexasaccharide
7 (1 eq)	**1** (1.2 eq)	$Et_2O \cdot BF_3$	−15 ~ 20 °C	7 d	not obtained
7 (1 eq)	**1** (1.2 eq)	Me_3SiOTf	−15 ~ 20 °C	24 h	not obtained

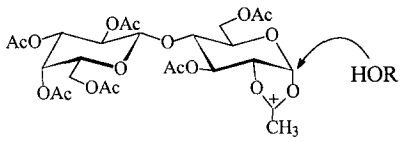
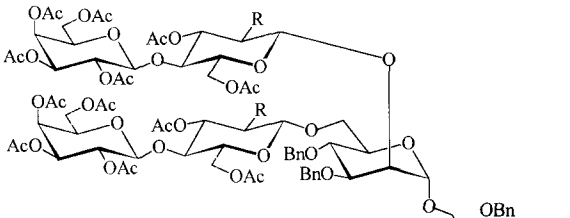
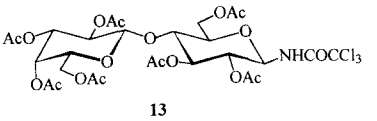


Fig. 1.

with **7** still failed to produce the corresponding hexasaccharide. The observable products were **13** and **14** when $Et_2O \cdot BF_3$ or Me_3SiOTf was used as activator.



	R	R'	R"
9	OAc	OCH_3	H
10	OAc	H	OCH_3
11	NPhth	OCH_3	H
12	NPhth	H	OCH_3



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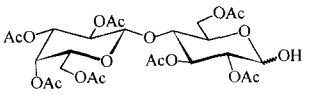

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Studies on the conformation of **7** *and* **8** *and discussion.*—Tetrasaccharides **7** and **8** differ only in the anomeric configuration of the reducing end methyl glycoside. Why do they behave completely differently in the attempted glycosylation with a same donor **1** under the same conditions? We reasoned that the steric hindrance difference between alcohol **7** and alcohol **8** in the transition state (Fig. 1) might be main cause for the unexpected difference in reactivity.

Nuclear magnetic resonance (NMR) spectroscopy is used to analyze the conformations of organic

Table 2
^1H NMR (500 MHz) and ^{13}C (125 MHz) NMR chemical shifts (ppm) and differences for tetrasaccharide **7** and tetrasaccharide **8**

	Tetrasaccharide **7**		Tetrasaccharide **8**		$\Delta\delta$ [δ(**7**)–δ(**8**)]	
	Proton(s)	Carbon	Proton(s)	Carbon	$\Delta\delta_H$	$\Delta\delta_C$
1	4.260	102.70	4.700	98.78	−0.440	3.92
2	3.670	74.17	3.781	74.77	−0.111	−0.60
3	3.904	74.26	3.920	73.92	−0.016	0.34
4	3.513	82.27	3.879	80.12	−0.366	2.15
5	3.336	75.32	3.663	73.92	−0.327	1.40
6	3.714	66.17	3.673	66.14	−0.041	0.03
	3.884		3.860		0.024	
1′	5.015	99.70	5.027	99.63	−0.012	0.07
2′	4.812	72.90	3.640	71.51	**1.172**	**1.39**
3′	4.086	67.54	4.078	67.70	0.008	−0.16
4′	3.799	79.03	3.797	79.01	0.002	0.02
5′	3.738	70.97	3.690	70.67	0.048	0.30
6′	3.650	68.14	3.654	68.15	−0.004	−0.01
	3.928		3.927		0.001	
1″	4.481	100.41	4.486	100.41	−0.005	0
2″	4.924	71.80	4.886	71.80	0.038	0
3″	5.122	72.96	5.132	72.93	−0.010	0.03
4″	3.781	76.18	3.792	76..16	−0.011	0.02
5″	3.493	72.43	3.503	72.41	−0.010	0.02
6″	4.051	62.07	4.073	62.05	−0.022	0.02
	4.451		4.463		−0.012	
1‴	4.458	101.05	4.470	101.03	−0.012	0.02
2‴	5.086	69.07	5.090	69.06	−0.004	0.01
3‴	4.941	70.97	4.908	70.95	0.033	0.02
4‴	5.325	66.61	5.324	66.59	0.001	0.02
5‴	3.854	70.60	3.884	70.59	−0.030	0.01
6‴	4.059	60.79	4.065	60.77	−0.006	0.02
	4.096		4.080		0.016	

molecules because atoms can be distinguished according to their chemical and geometrical environments. The structures of tetrasaccharides **7** and **8** are very similar. The difference between their ^{1}H- or ^{13}C-chemical shifts gives some information on conformation. Their ^{1}H- and ^{13}C-chemical shifts were assigned by the combined use of various NMR techniques, including $^{1}H-^{1}H$ COSY, $^{13}C-^{1}H$ COSY, HMBC and TOCSY (Table 2). The complete assignments were described in detail in a previously published paper [6].

It is understood that chemical-shift changes for H-1, H-4, H-5, C-1, C-4 and C-5 result mainly from the anomeric effect at C-1. However, this does not explain the chemical-shift changes for H-2′ and C-2′. In both series, the H-2′ signal of compound **7** is shifted 1.172 ppm downfield while the C-2′ signal of compound **7** is shifted 1.389 ppm downfield relative to compound **8**. The explanation probably stems from the deshielding effect of benzyl group close to H-2′ and C-2′ in compound **7**.

The favored conformations of **7** and **8** presented in Fig. 2 were modeled using Discover Software (BIOSYM). These conformations demonstrate that the steric hindrance around HO-2′ of alcohol **7** is greater than the steric hindrance around HO-2′ of alcohol **8**. Consequently the steric hindrance around HO-2′ of the tetrasaccharide acceptor **7** leads to higher potential energy in the transition state for glycosylation, which prevents the condensation of **1** and **7**.

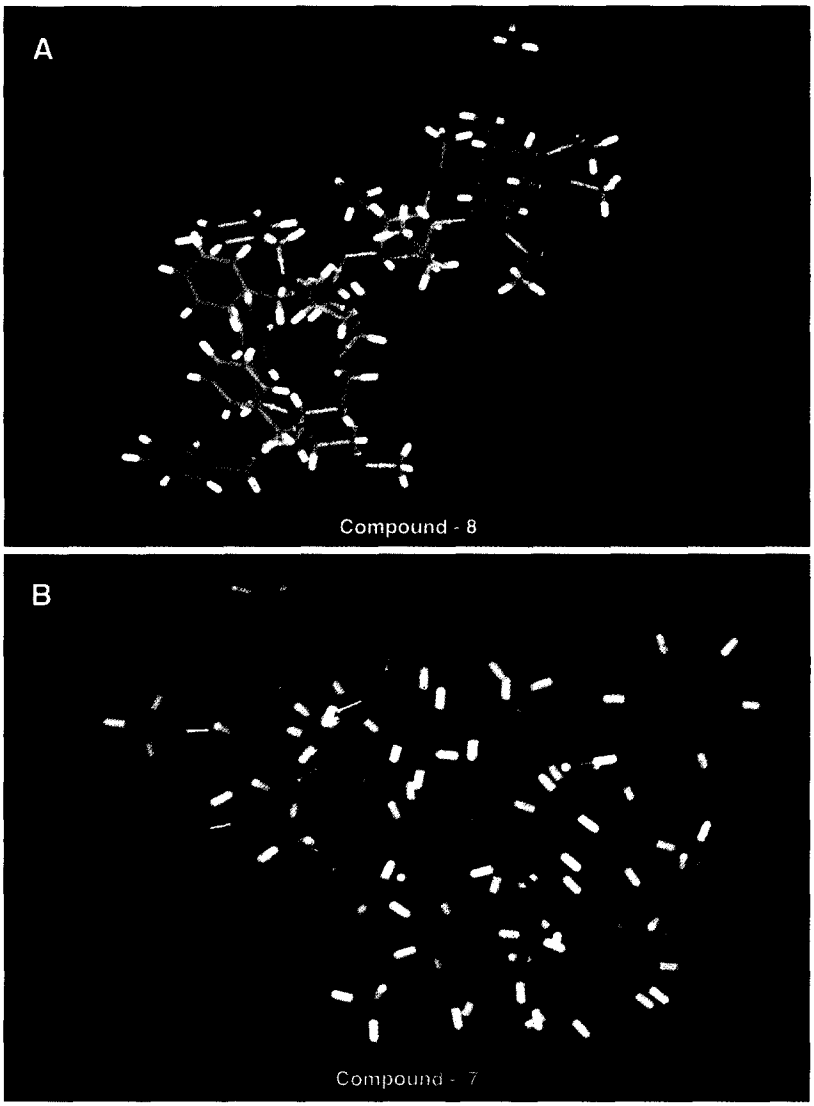

Fig. 2. (A) The favored conformation of compound **8**. (B) The favored conformation of compound **7**.

3. Experimental

General methods.—Optical rotations were determined with a Perkin–Elmer Model 241 MC polarimeter at 15 °C. Column chromatography was performed on silica gel H (Qingdao) and fractions were monitored by TLC on silica gel GF_{254} (Qingdao). Detection was effected by examination under UV light and by charring with 5% phosphomolybdic acid hydrate in EtOH. Elemental analyses were performed on a Perkin–Elmer 240C instrument. ^1H NMR spectra were recorded at 300 MHz with a Bruker AM-300 and at 500 MHz with a Bruker AM-500 apparatus at 25 °C. ^{13}C NMR spectra were recorded at 75 MHz with a Bruker AM-300 and at 125 MHz with a Bruker AM-500 apparatus at 25 °C. The values of δ_H and δ_C are expressed in ppm downward from the signal for internal Me_4Si for solutions in $CDCl_3$.

Methyl 2,6-di-O-acetyl-3,4-di-O-benzyl-α-D-mannopyranosyl-(1 → 6)-(2,3,4-tri-O-benzyl-β-D-mannopyranoside) (**4**).—A mixture of **2** (1.36 g, 2.20 mmol), **3** (0.82 g, 1.77 mmol) in dry CH_2Cl_2 (9 mL) was stirred with powdered molecular sieves (4 Å, 0.1 g) for 2 h. To this stirred mixture was added dropwise Me_3SiOTf (21 μL Me_3SiOTf in 1.2 mL CH_2Cl_2) and the mixture was stirred for 1.5 h at 0 °C. The acid was neutralized with Et_3N, and the mixture filtered and concentrated in vacuo. Column chromatography of the residue on silica gel gave **4** (1.42 g, 90%) as a colorless syrup: $[\alpha]_D$ −45° (*c* 1, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 5.38 (d, *J* 3.6 Hz, 1 H, H-1′), 4.17 (d, *J* 3.3 Hz, 1 H, H-1), 3.40 (s, 3 H, OCH_3), 2.05 and 1.95 (s, each 3 H, 2 Ac); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.83 and 170.17 (2 C=O), 102.82 (C-1), 97.8 (C-1′), 57.21 (OCH_3), 21.11 and 20.87 (2 Ac). Anal Calcd for $C_{52}H_{58}O_{13}$: C, 70.10; H, 6.56. Found: C, 70.03; H, 6.55.

Methyl 3,4-di-O-benzyl-α-D-mannopyranosyl-(1 → 6)-(2,3,4-tri-O-benzyl-β-D-mannopyranoside) (**5**).—Compound **4** was stirred with NaOMe in MeOH (5 mL, pH 10) for 30 h at room temperature. Then the solution was neutralized with 732 (H^+) cation-exchange resin, filtered and concentrated to dryness to afford **5** as white solid (1.2 g, 95%): $[\alpha]_D$ −45° (*c* 1, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 4.92 (d, *J* 1.1 Hz, 1 H, H-1′), 4.19 (s, 1 H, H-1), 3.41 (s, 3 H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): 102.82 (C-1), 99.67 (C-1′), 57.20 (OCH_3). Anal Calcd for $C_{48}H_{54}O_{11}$: C, 71.45; H, 6.74. Found: C, 71.42; H, 6.79.

Methyl (2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-(2,3,6-tri-O-acetyl-β-D-glucopyranosyl)-(1 → 6)-(3,4-di-O-benzyl-α-D-mannopyranosyl)-(1 → 6)-(2,3,4-tri-O-benzyl-β-D-mannopyranoside) (**7**).—A mixture of **1** (0.86 g, 1.10 mmol), **5** (740 mg, 0.92 mmol) and powdered molecular sieves (4 Å, 0.2 g) in dry CH_2Cl_2 (3 mL) was stirred for 3 h at room temperature and cooled to −15 °C. Then $Et_2O \cdot BF_3$ (189 μL) in CH_2Cl_2 (1.5 mL) was added dropwise. Cooling was removed and the mixture was stirred at room temperature overnight. The acid was neutralized with $NaHCO_3$ (200 mg), filtered and concentrated in vacuo. Column chromatography (10:3, 3:1, 9:4 petroleum ether–acetone) of the residue on silica gel gave **7** (0.74 g, 57%) as a white solid: $[\alpha]_D$ −29° (*c* 1, $CHCl_3$); R_f 0.32 (3:2 petroleum ether–acetone); ^1H and ^{13}C NMR (Table 2). Anal. Calcd for $C_{74}H_{88}O_{28}$: C, 62.35; H, 6.22. Found: C, 62.42; H, 6.29.

Methyl (2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-(2,3,6-tri-O-acetyl-β-D-glucopyranosyl)-(1 → 6)-(3,4-di-O-benzyl-α-D-mannopyranosyl)-(1 → 6)-(2,3,4-tri-O-benzyl-α-D-mannopyranoside) (**8**).—Condensation of **1** (250 mg, 0.32 mmol) with **6** (220 mg, 0.27 mmol) in CH_2Cl_2 (3 mL) in the presence of $Et_2O \cdot BF_3$ (50 μL $Et_2O \cdot BF_3$ in 1 mL CH_2Cl_2) was carried out by the procedures described for the preparation of **7**, affording **8** (280 mg, 73%) as white solid. $[\alpha]_D$ +37° (*c* 1, $CHCl_3$); R_f 0.35 (3:2 petroleum ether–acetone); ^1H and ^{13}C NMR (Table 2). Anal. Calcd for $C_{74}H_{88}O_{28}$: C, 62.35; H, 6.22. Found: C, 62.30; H, 6.30.

Methyl (2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-(2,3,6-tri-O-acetyl-β-D-glucopyranosyl)-(1 → 2)-[(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-(2,3,6-tri-O-acetyl-β-D-glucopyranosyl)-(1 → 6)]-(3,4-di-O-benzyl-α-D-mannopyranosyl)-(1 → 6)-(2,3,4-tri-O-benzyl-α-D-mannopyranoside) (**9**).—A mixture of **1** (130 mg, 0.17 mmol), **8** (200 mg, 0.14 mmol) and powdered molecular sieves (4 Å, 100 mg) in dry CH_2Cl_2 (20 mL) was stirred for 3 h at room temperature and cooled with ice-salt bath. Then $Et_2O \cdot BF_3$ (0.14 ml of 1 M solution) was added. The mixture was allowed to attain room temperature slowly and was stirred another 12 h. The acid was neutralized with $NaHCO_3$ (0.6 g), the solid was filtered off and washed with CH_2Cl_2 (3 × 10 mL). The combined organic layer was concentrated in vacuo. Column chromatography (10:3, 3:1, 9:4 petroleum ether–acetone) of the residue on silica gel gave **9** (186 mg) in 65% yield. The physical data and NMR data were agreed with these of the reported compound [3] which was synthesized by another route.

Acknowledgements

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