A General Synthesis of N-Glycosides. III.[1,2] A Simple Synthesis of Pyrimidine Disaccharide Nucleosides

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Silylated uracils as well as 6-azauracils react smoothly with peracetylated reducing disaccharides in the presence of $SnCl_4$ to give the corresponding disaccharide nucleosides. Preliminary experiments show that tri- and tetrasaccharides react analogously.

Wolfrom and coworkers[3] have described the synthesis of acetylated purine disaccharide nucleosides *via* the chloromercuric salts, which gave the pure crystalline 9β-disaccharide purine nucleosides in ~10–30% overall yields. More recently Rao and Lerner[4] prepared analogously 1β-cellobiosyl- and 1β-lactosylbenzimidazole, but, to our knowledge, no disaccharide pyrimidine nucleosides have as yet been prepared starting from disaccharides prior to our own work.

Thus we wondered whether the readily available peracetylated oligosaccharides with a reducing end group, especially acetylated disaccharides like octaacetylcellobiose, -lactose, and -maltose, would not react with silylated pyrimidines and $SnCl_4$ to give the corresponding pyrimidine disaccharide nucleosides. We expected a smooth reaction since it is well known[5] that Friedel–Crafts catalysts like $SnCl_4$ or $TiCl_4$ cleave the 1-O–acetyl bond in these acetylated oligosaccharides in preference to the glycosidic linkage between the sugar residues to give a C-1 cation.

However, in our initial reactions of crystalline disaccharide octaacetates with silylated pyrimidines and $SnCl_4$ the yields of acetylated pyrimidine disaccharide nucleosides varied widely and several other products were formed.

A detailed investigation of the reaction of octaacetylcellobiose (**1**) with silylated 6-azauracil (**2**) revealed that commercial as well as our own samples of **1** always contained up to 0.5% ethanol or other alcohols from recrystallization. Since alcohols and acetic acid react with $SnCl_4$ or $TiCl_4$ to give HCl,[2] the disaccharide bond in **1** or in the already formed disaccharide nucleoside **3** was cleaved. Thus not only **3** but also the 6-azauracil *N*-glucosides **4** and **5** were formed by the reaction of **2** with the cations obtained by the indicated types of glycosidic bond cleavage of **1** and **3** (Scheme I).

The nucleoside **4** was identified by tlc comparison with an authentic sample. Furthermore both acetylated nucleosides **4** and **5** gave on saponification the known free 2-(β-D-glucopyranosyl)-2,3,4,5-tetrahydro-1,2,4-triazine-3,5-dione (**8**),[1] thus making the structure of **5** highly probable.

To avoid formation of HCl the acetylated cellobiose was therefore pulverized and dried at elevated temperature in high vacuum or by azeotropic distillation. Repetition of the reaction of dried **1** with **2** and $SnCl_4$ in boiling 1,2-dichloroethane now gave the crystalline acetylated disaccharide nucleoside **3** in 80% yield as the only product.

Reaction of **2** with hepta-*O*-acetylcellobiosyl chloride[5] in the presence of $SnCl_4$ in 1,2-dichloroethane gave beside **3** the N_3- as well as the N_1,N_3-bis(disaccharide) nucleoside.[6]

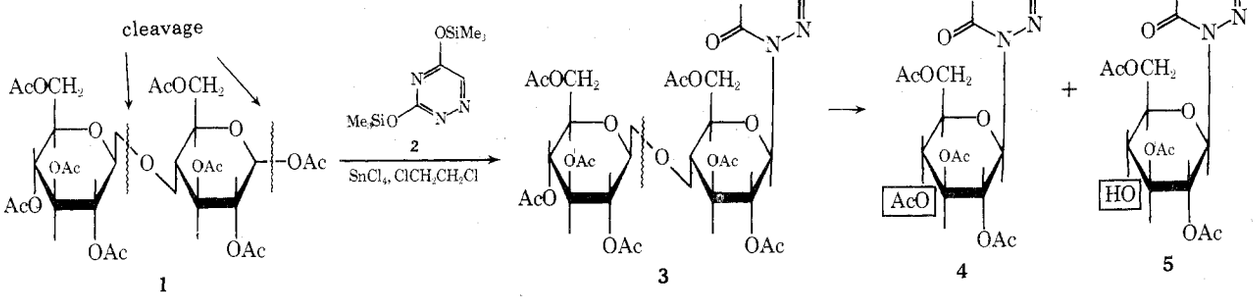
In most subsequent experiments the carefully dried peracetylated disaccharides were treated with silylated pyrimidines and $SnCl_4$ in boiling 1,2-dichloroethane yielding 60–80% of the corresponding peracetylated disaccharide nucleosides, which often either crystallized spontaneously or after purification over a column of alumina. Methanolysis with sodium methylate in methanol gave in high yields the free disaccharide nucleosides.

The structures of the acetylated disaccharide nucleosides are supported by their mass spectra as exemplified by the fragmentation of the maltose nucleoside **6** (Scheme II). Further obvious fragments observed were *m/e* 744 (M − H), 685 (M − CH_3COOH), 684 (744 − CH_3COOH), 625 (M − 2 CH_3COOH), 624 (744 − 2CH_3COOH).

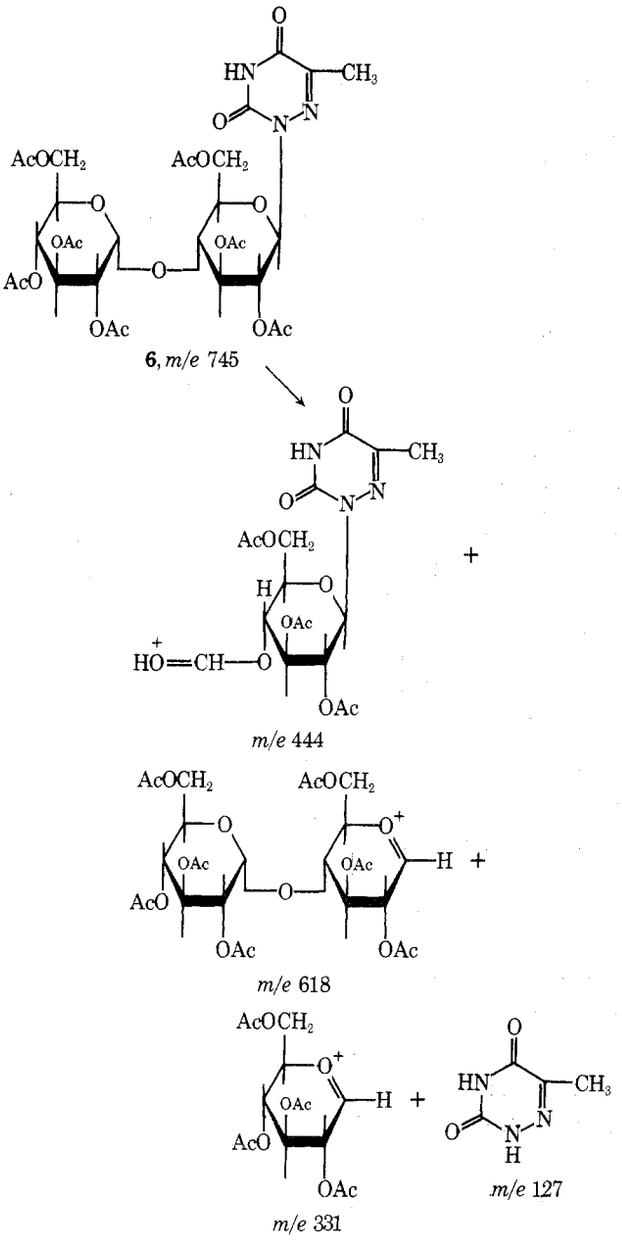
An additional proof for the structure of the free disaccharide nucleosides was provided when the free disaccharide nucleoside **7a** with an α-glucosidic bond was incubated with maltase (α-glucosidase) in aqueous buffer solution to give the glucoside **8** in practically quantitative yield, which was identical with an authentic sample (Scheme III).

Since peracetylated tri- and tetrasaccharides are not commercially available we prepared and reacted a mixture of peracetylated cellotriose (**9**) and cellotetraose (**10**) with silylated 6-azauracil (**2**) and obtained a mixture of the acetylated oligosaccharide nucleosides **11** and **12** in ~70% yield, which were separated by preparative tlc, and the

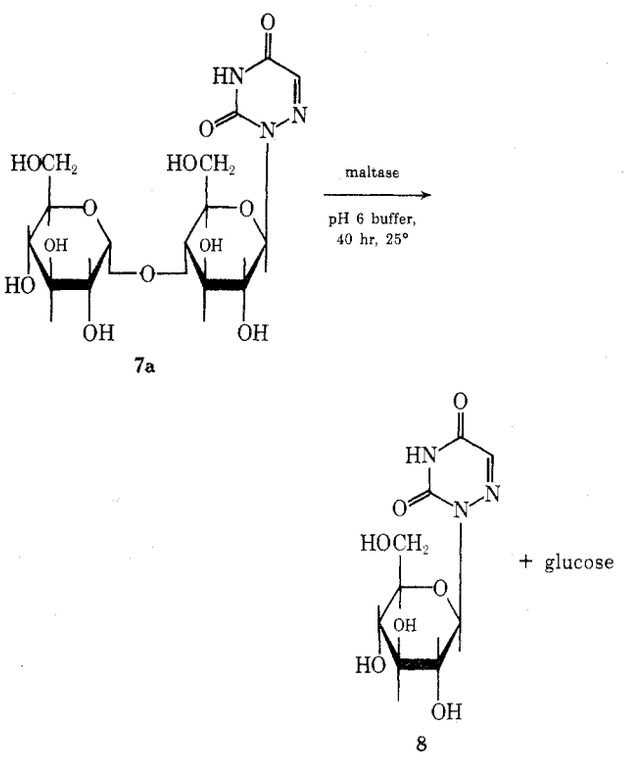
Scheme I



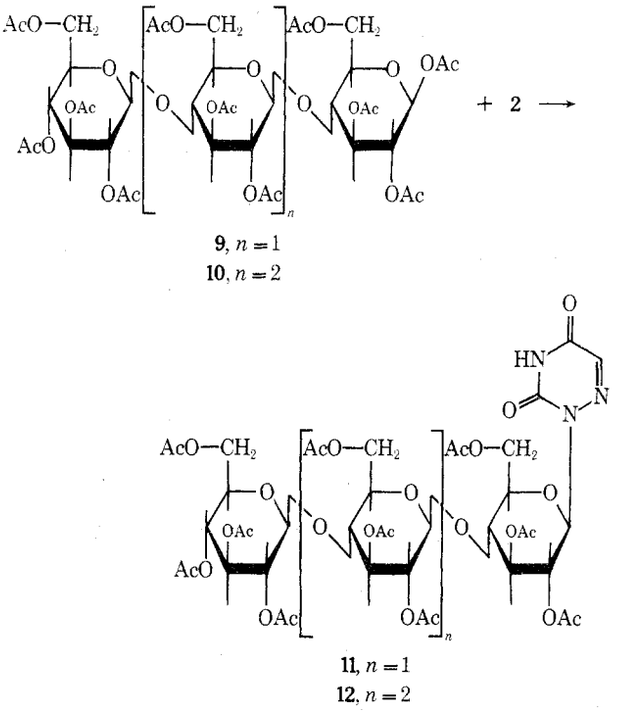
Scheme II



Scheme III



Scheme IV



structures were assigned by nmr ratio of H-5 and H-1′ to acetyl CH_3 (Scheme IV).

In Tables I and II the formation of acetylated and free disaccharide nucleosides is summarized. Besides the already mentioned octaacetylcellobiose (**1**), octaacetylmaltose (**13**) and octaacetyllactose (**14**) were treated with 3,5-bis(trimethylsilyloxy)-1,2,4-triazine (**2**), 6-methyl-3,5-*S,O*-bis(trimethylsilyl)-1,2,4-triazine (**15**), 6-methyl-3,5-bis(trimethylsilyloxy)-1,2,4-triazine (**16**), 5-ethyl-2,4-bis-(trimethylsilyloxy)pyrimidine (**17**), 2,4-bis(trimethylsilyloxy)pyrimidine (**18**), and 2,4-*O,N*-bis(trimethylsilyl)-2-hydroxy-4-aminopyrimidine (**28**).

Experimental Section

For instruments and the purification of solvents compare part I of this series.[2]

Tlc systems follow: system A [toluene–acetic acid–H_2O (5:5:1)];[7] system B (ethyl acetate); system C [ethyl acetate–hexane (2:1)]; system D [*n*-butyl acetate–methyl glycol–H_2O (4:1:2)]; and system E [1-propanol–aqueous NH_3 (7:3)]. For the column chromatography neutral alumina (Woelm) and standard silica gel Merck (Darmstadt) were used.

Preparation of the Acetylated Disaccharides. The acetylated disaccharides were prepared according to standard methods and dried either by heating 1–2 hr to 80–100° (10^{-3} mm) or by azeotropic distillation with 1,2-ethylene chloride: Octaacetyl-α-cellobiose,[8] octaacetyl-β-maltose,[9] octaacetyl-β-lactose[10] were obtained.

Preparation of Acetylated Oligosaccharides. The acetolysis of cellulose was performed according to Hess and Dziengel.[11] The crude product was dried *in vacuo* at 50° for 2 days and extracted with hot methanol several times. The methanolic solution was concentrated *in vacuo*. On stirring over night crystals of octaacetyl-α-cellobiose (**1**, mp 222–223°) had formed, which were filtered.

Table I

Silylated pyrimidine	Peracetyled disaccharide	Protected nucleoside Yield, %[a]	Mp,[b] °C	$[\alpha]^{20}D$[c]	H-1',[d]	Formula (mol wt)	Analysis, % calcd (found) C	H	N	S
2	1	3 80	241–242	−59.0	5.88	$C_{29}H_{37}N_3O_{19}$ (731.64)	47.61 (47.50)	5.10 (5.31)	5.74 (6.03)	
2	13	7 72	130–132	−31.5	5.88	$C_{29}H_{37}N_3O_{19}$ (731.64)	47.61 (47.40)	5.10 (5.48)	5.74 (5.93)	
15	1	19 95		−17.1	6.61	$C_{30}H_{39}N_3O_{18}S$ (761.73)	47.31 (47.02)	5.16 (5.46)	5.51 (5.56)	4.21 (4.20)
16	1	20 63	150–153	−48.0	5.73	$C_{30}H_{39}N_3O_{19}$ (745.66)	48.32 (48.02)	5.27 (5.40)	5.64 (5.63)	
15	13	21 70	181–182	−68.9	6.60	$C_{30}H_{39}N_3O_{18}S$ (761.73)	47.31 (47.02)	5.16 (5.50)	5.51 (5.61)	4.21 (4.11)
16	13	22 63	125–126	−35.7	5.86	$C_{30}H_{39}N_3O_{19}$ (745.66)	48.32 (47.97)	5.27 (5.49)	5.64 (5.51)	
2	14	23 80		−29.6	5.91	$C_{29}H_{37}N_3O_{19}$ (731.64)	47.61 (47.67)	5.10 (5.38)	5.74 (5.56)	
15	14	24 66		−3.1	6.61	$C_{30}H_{39}N_3O_{18}S$ (761.73)	47.31 (47.34)	5.16 (5.39)	5.51 (5.72)	4.21 (4.17)
16	14	25 69		−31.5	5.75	$C_{30}H_{39}N_3O_{19}$ (745.66)	48.32 (48.07)	5.27 (5.33)	5.64 (5.65)	
17	1	26 78			5.80	$C_{32}H_{42}N_2O_{19}$ (758.71)	50.66 (50.39)	5.58 (5.76)	3.69 (3.61)	
18	13	27 71			5.85	$C_{30}H_{38}N_2O_{19}$ (730.81)	49.31 (49.06)	5.34 (5.39)	3.86 (3.75)	
28	14	29 81		9.1	6.15	$C_{30}H_{39}N_3O_{18}$ (729.67)	49.38 (49.19	5.39 (5.55)	5.76 (5.61)	

[a] According to standard procedure. [b] From ethanol. [c] *c* 1, $CHCl_3$. [d] $CDCl_3$, parts per million (d, J = 9 Hz).

Table II

Protected nucleoside	Free nucleoside Yield, %[a]	Mp,[b] °C	$[\alpha]^{20}D$[c]	H-1',[d]	Formula (mol wt)	Analysis, % calcd (found) C	H	N	S
3	3a 95	202–205	−39.0	5.63	$C_{15}H_{23}N_3O_{12}\cdot H_2O$ (455.50)	39.55 (39.74)	5.53 (5.84)	9.22 (8.96)	
7	7a 75	188–190	−47.3	5.66	$C_{15}H_{23}N_3O_{12}\cdot H_2O$ (455.50)	39.55 (39.84)	5.53 (5.76)	9.22 (8.97)	
19	19a 74	274–275[e]	−18.8	6.65	$C_{16}H_{25}N_3O_{11}S$ (467.47)	41.11 (41.02)	5.39 (5.48)	8.99 (8.81)	6.86 (6.68)
20	20a 82	260[e]	−33.3[f]	5.65	$C_{16}H_{25}N_3O_{12}$ (451.40)	42.57 (42.83)	5.59 (5.82)	9.31 (9.07)	
21	21a 65	207–209	−60.8	6.63	$C_{16}H_{25}N_3O_{11}S\cdot CH_3OH$ (499.62)	40.87 (40.60)	5.88 (5.93)	8.45 (8.35)	6.59 (6.49
22	22a 79	174–175	−55.7[f]	5.65	$C_{16}H_{25}N_3O_{12}$ (451.40)	42.57 (42.43)	5.59 (5.71)	9.31 (9.19)	
23	23a 72	292–294	−24.3	5.68	$C_{15}H_{23}N_3O_{12}$ (437.38)	41.19 (41.17)	5.30 (5.44)	9.61 (9.47)	
24	24a 83	218–221	−10.0	7.30[g]	$C_{16}H_{25}N_3O_{11}S$ (467.47)	41.11 (40.84)	5.39 (5.56)	8.99 (8.84)	6.86 (6.72)
25	25a 78	256–258[e]	−20.8[f]	5.65	$C_{16}H_{25}N_3O_{12}$ (451.40)	42.57 (42.84)	5.59 (5.84)	9.31 (9.18)	
26	26a				$C_{18}H_{28}N_2O_{12}$ (464.42)	46.55	6.08	6.03	
27	27a				$C_{16}H_{24}N_2O_{12}$ (436.37)	44.03	5.54	6.42	
29	29a 90		11.0[f]	5.70	$C_{16}H_{25}N_3O_{11}$ (435.40)	44.14 (43.86)	5.79 (5.93)	9.65 (9.49)	

[a] According to standard procedure. [b] From ethanol–H_2O. [c] *c*, 0.4 (ethanol–H_2O, 3:1). [d] D_2O, parts per million (d, J = 9 Hz). [e] Decomposition. [f] *c*, 0.4 (ethanol–H_2O, 3:2). [g] In pyridine-d_5.

The residual oil gave on methanolysis[12] the free oligosaccharides. The methanolic solution was neutralized with H_2SO_4 and evaporated *in vacuo* to dryness. The aqueous extract[13] was reacetylated according to Hess and Dziengel[11] with acetic anhydride–pyridine to give the acetates, which were separated by column chrmatography on silica gel eluting with mixtures of ether–acetone.

The purity of the oligosaccharide fractions was checked by tlc (system A). The spots were detected by spraying with H_2SO_4 and heating to 110° for 5 min. For the silyl Hilbert–Johnson reaction a chromatography fraction was used, which according to tlc (system A) consisted of ~60% hendecaacetylcellotriose (**9**), tetradecaacetylcellotetraose (**10**), and some octaacetylcellobiose (**1**).

Preparation of the Heterocycles. The 1,2,4-triazine derivatives were prepared according to Gut[14] and silylated according to standard methods.[2]

A. General Procedure for the Preparation of Disaccharide Nucleosides. To a solution of peracetylated disaccharide (15 mmol) and of the persilylated pyrimidine (16.5 mmol) in 1,2-dichloroethane (150 ml) $SnCl_4$ (1.26 ml, 10.8 mmol) in 1,2-dichloroethane (20 ml) was added. The reaction mixture was either refluxed for 2 hr under exclusion of moisture (preparation of **3, 7, 14–20, 29**) or kept for 4 hr at 60° (preparation of **21, 22**). The cooled solution was diluted with methylene chloride (200 ml) and poured into ice–saturated $NaHCO_3$ solution (200 ml). The organic layer was separated and the aqueous solution was washed with methylene chloride (100 ml). The combined organic solution was filtered through sand–Celite, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was chromatographed on neutral alumina (activity III) and eluted with ethyl acetate–methanol.

B. General Procedure for the Methanolysis of Acetylated Disaccharide Nucleosides. Acetylated disaccharide nucleoside (4.4 mmol) was suspended in dry methanol (20 ml) and 1 *N* $NaOCH_3$ solution (8.8 mmol) was added whereupon the substance dissolved and after 30–40 min a white precipitate was formed.

After stirring the reaction solution overnight at room temperature the alkaline solution was neutralized by addition of wet Dowex 50 H^+, whereupon the precipitate dissolved. The resin was filtered and washed with methanol and water. After evaporation of the combined filtrates the residue was crystallized from ethanol or a mixture of ethanol–water.

2-(Hepta-*O*-acetyl-β-D-cellobiosyl)-2,3,4,5-tetrahydro-1,2,-4-triazine-3,5-dione (3). On reaction of impure **1** (containing alcohol) the crude reaction mixture showed on tlc evaluation (system B) besides **3** a number of less polar products, one of which was identical with an authentic sample of **4**. A mixture of **4** and the slightly slower moving **5** was isolated by preparative tlc (system B) and saponified with methanolic ammonia to give a product which gave only a single spot on tlc (system E) identical with an authentic sample of **8**.

Reaction of Hepta-*O*-acetylcellobiosyl Chloride with 2. To a chilled solution of hepta-*O*-acetylcellobiosyl chloride (2.5 g, 3.8 mmol) and **2** (4.5 mmol) in dry 1,2-dichloroethane (30 ml), $SnCl_4$ (0.1 ml, 0.86 mmol) in 1,2-dichloroethane (5 ml) was added. After 4 days at 22° and work-up the crude product contained according to tlc examination (silica gel, system C) at least two products. The nucleosides were separated by fractional precipitation from ethanol. On dissolving the crude reaction product in a small volume of hot ethanol a precipitate of pure **3** was obtained. Further fractional precipitation gave the N_4- as well as the N_2,N_4-bisglycoside.

Yield of **3**: 1.68 g (60.5%); λ_{max} (CH_3OH) 263 nm, λ_{max} (CH_3OH + NaOH) (0.1 *N*) 262 nm.

4-(Hepta-*O*-acetyl-β-D-cellobiosyl)-2,3,4,5-tetrahydro-1,2,4-triazine-3,5-dione: yield, 0.25 g (8.9%); λ_{max} (CH_3OH) 259 nm, λ_{max} (CH_3OH + NaOH) (0.1*N*) 305 nm.

2,4-Bis(hepta-*O*-acetyl-β-D-cellobiosyl)-2,3,4,5-tetrahydro-1,2,4-triazine-3,5-dione: yield, 0.148 g (5.3%); λ_{max} (CH_3OH) 263 nm, λ_{max} (CH_3OH + NaOH) (0.1 *N*) 262 nm; nmr ($CDCl_3$) δ 7.35 (s, 1, H-5), 5.83 (m, 2, H-1′N_4, H-1′ N_2), 2.2–1.8 (m, 42, OAc).

Enzymatic Cleavage. 2-(β-D-Maltosyl)-2,3,4,5-tetrahydro-1,2,4-triazine-3,5-dione (**7a**, 60 mg, 0.37 mmol) was dissolved in acetate buffer, pH 6 (10 ml, 0.1 *N*). After addition of α-glucosidase (2 mg, Boehringer-Mannheim, suspension in 3.2 *m* ammonium sulfate solution, pH ~6, 1 ml) the reaction was incubated at 25° and the enzymatic cleavage was followed by tlc. After 40 hr all starting material had disappeared and a new product had formed, which was identical in polarity with 2-(β-D-glucopyranosyl)-2,3,4,5-tetrahydro-1,2,4-triazine-3,5-dione (**8**). The solution was filtered over a column of Dowex 50 H^+ and the column was washed with methanol–H_2O. Filtrate and washings were combined and evaporated *in vacuo* to dryness. Preparative tlc on silica gel (system E) gave a fluorescent band which was eluted with methanol–H_2O and crystallized from ethanol to give 35.9 mg (95.1%) of **8** as white needles, mp 211–212° (lit.[2] 210–212°).

Reaction of a Mixture of Hendecaacetylcellotriose (9) and Tetradecaacetylcellotetraose (10) with 2. To a mixture of **9, 10,** and some **1** (2.0 g) and **2** (8 mmol) in 1,2-dichloroethane (100 ml), $SnCl_4$ (0.8 ml, 6.8 mmol) was added. After 2 hr of reflux and work-up (2.1 g colorless foam) an aliquot of the mixture of **11** and **12** (1.6 g) was subjected to preparative tlc (silica gel, system D) to give the nucleosides **11** and **12**.

2-(Deca-*O*-acetyl-β-D-cellotriosyl)-2,3,4,5-tetrahydro-1,2,4-triazine-3,5-dione (**11**): nmr ($CDCl_3$) δ 7.39 (s, 1, H-5), 5.84 (d, 1, *J* = 9 Hz, H-1′), 2.2–1.9 (m, 30, OAc).

2-(Trideca-*O*-acetyl-β-D-cellotetraosyl)-2,3,4,5-tetrahydro-1,2,4-triazine-3,5-dione (**12**): nmr ($CDCl_3$) δ 7.39 (s, 1, H-5), 5.83 (d, 1, *J* = 9 Hz, H-1′), 2.2–1.8 (m, 39, OAc).

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Registry No.—**1,** 5346-90-7; **2,** 17331-61-2; **3,** 38909-08-9; **3a,** 38909-17-0; **7,** 38909-10-3; **7a,** 38909-16-9; **9,** 31873-41-3; **10,** 52523-12-3; **11,** 39020-67-2; **12,** 52523-13-4; **13,** 20880-60-8; **14,** 52554-32-2; **15,** 52571-10-5; **16,** 17331-64-5; **17,** 31167-05-2; **18,** 10457-14-4; **19,** 52523-14-5; **19a,** 52523-15-6; **20,** 38909-07-8; **20a,** 38909-15-8; **21,** 38909-09-0; **21a,** 38909-18-1; **22,** 38909-06-7; **22a,** 38909-14-7; **23,** 38909-11-4; **23a,** 38909-19-2; **24,** 38909-12-5; **24a,** 38909-20-5; **25,** 52523-16-7; **25a,** 52523-17-8; **26,** 38909-23-8; **26a,** 38909-24-9; **27,** 52523-18-9; **27a,** 52523-19-0; **28,** 18037-10-0; **29,** 52523-20-3; **29a,** 52523-21-4.

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