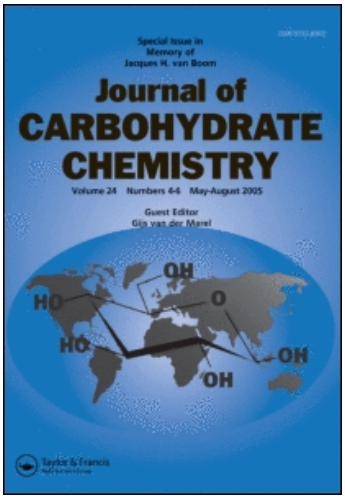

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p-Nitrophenyl Glycosides of Chitooligosaccharides; Their Syntheses Through the Autocatalytic Fusion Reaction of Chitooligosaccharides Acetates with p-Nitrophenol

Fumio Nanjo[a]; Kazuo Sakai[a]; Taichi Usui[b]; Izumi Takai[c]; Yoshiharu Ishido[c]
[a] NFI Institute, Kogawa-shinmachi, Yaizu, Shizuoka, Japan [b] Department of Agricultural Chemistry, Faculty of Agriculture, Shizuoka University, Ohya, Shizuoka, Japan [c] Department of Chemistry, Faculty of Science, Tokyo Institute of Technology, O-okayama, Meguro-ku, Tokyo, Japan

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p-NITROPHENYL GLYCOSIDES OF CHITOOLIGOSACCHARIDES; THEIR SYNTHESES THROUGH THE AUTOCATALYTIC FUSION REACTION OF CHITOOLIGOSACCHARIDES ACETATES WITH p-NITROPHENOL

Fumio Nanjo,[*1] Kazuo Sakai,[*1] Taichi Usui,[*2] Izumi Takai, and Yoshiharu Ishido

[*1]NFI Institute, Kogawa-shinmachi, Yaizu, Shizuoka, 425 Japan
[*2]Department of Agricultural Chemistry, Faculty of Agriculture, Shizuoka University, Ohya, Shizuoka, 422 Japan
Department of Chemistry, Faculty of Science, Tokyo Institute of Technology, O-okayama, Meguro-ku, Tokyo, 152 Japan

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ABSTRACT

Fusion reactions of p-nitrophenol with acetates of chitobiose, chitotriose, chitotetraose, and chitopentaose under autocatalytic conditions gave the corresponding p-nitrophenyl chitooligosides acetates in acceptable yields. Complete O-deacetylation of these acetates was attained by treatment with sodium methoxide in methanol under reflux in the case of the latter two, and at room temperature with the former two.

INTRODUCTION

The autocatalytic fusion reaction of a sugar peracetate with a protic nucleophile, e.g., 2,6-dichloropurine[1,2] and p-nitrophenol,[1,2] has been reported to be extremely simple to perform to give the corresponding glycosyl compounds in good yields.

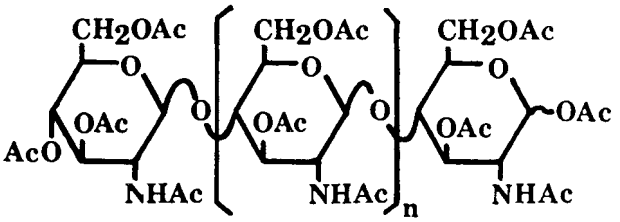
The anomeric p-nitrophenyl glycosides of a number of chitooligosaccharides have been synthesized by the coupling reaction in a solvent between sodium p-nitrophenoxide and the corresponding chitooligosyl chlorides. The p-nitrophenyl glycosides of the chitooligosaccharides, p-nitrophenyl 2-

acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (9),[3] (i.e., N,N'-diacetyl-β-chitobioside), 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**10**),[4] (i.e., N,N',N"-triacetyl-β-chitotrioside), and 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**11**)[5] (i.e., N,N',N",N"'-tetraacetyl-β-chitotetraoside) were performed in this way. These chitooligosaccharides are of interest as potential substrates for egg-white lysozyme.

The simple procedure involved in the fusion method led us to explore its application to the synthesis of a series of p-nitrophenyl glycosides of chitooligosaccharides, in anticipation that we might be able to prepare a substrate more susceptible to lysozyme. We now report the results obtained herein.

RESULTS AND DISCUSSION

Hydrochlorides of chitobiose, chitotriose, chitotetraose, and chitopentaose were prepared by hydrolysis of chitosan.[6] These chitooligosaccharide hydrochlorides were respectively converted to the corresponding peracetates by treatment with acetic anhydride - fused zinc chloride, giving chitobiose octaacetate (**1**; 77% yield), chitotriose hendecaacetate (**2**; 89% yield), chitotetraose tetradecaacetate (**3**; 95% yield), and chitopentaose heptadecaacetate (**4**; 88% yield) as mixtures of their anomers (ca. 1:1). The β-anomer of a sugar peracetate is very susceptible to the autocatalytic reaction in contrast to the corresponding α-anomer. For example, the autocatalytic fusion reaction of 2-acetamido-2-deoxy-1,3,4,6-tetra-O-acetyl-β-D-glucopyranose and p-nitrophenol gave only the corresponding β-glycoside, but the α-anomer peracetate gave no glycoside species. Therefore, we attempted, but were unsuccessful in establishing an efficient method for the synthesis of peracetates of a chitooligosaccharide containing β-anomer preponderantly, using chitobiose and chitotriose as model compounds. The method used for the



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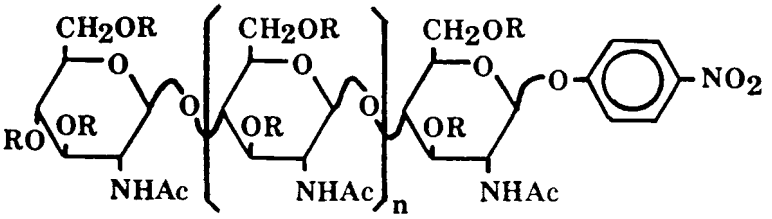

5: n=0, R=Ac 6: n=1, R=Ac

7: n=2, R=Ac 8: n=3, R=Ac

9: n=0, R=H 10: n=1, R=H

11: n=2, R=H 12: n=3, R=H

preparation of 2-acetamido-1,3,4,6-tetra-O-acetyl-β-D-glucopyranose, involving treatment of 2-amino-2-deoxy-D-glucopyranose hydrochloride with diethylamine, followed by acetylation with acetic anhydride in pyridine,[7] gave an α/β ratio of about 1:1 on application to chitobiose and chitotriose. Acetylation of chitotriose with acetic anhydride in the presence of sodium acetate gave an α/β mixture of 4:1.

The peracetates prepared by the acetic anhydride - zinc chloride procedure were then subjected to fusion reaction with p-nitrophenol under the autocatalytic conditions (180 - 190 °C) and gave the corresponding β-glycosides, respectively, in an acceptable yield, i.e., p-nitrophenyl heptaacetyl-β-chitobioside (5; 24% yield), decaacetyl-β-chitotrioside (6; 32% yield), tridecaacetyl-β-chitotetraoside (7; 27% yield), and hexadecaacetyl-β-chitopentaoside (8; 21% yield). Com-

pound **8** is a new compound. The yields obtained are reasonable since products are formed only from the β-peracetates in the 1:1 α/β mixture of starting chitooligosaccharides percetates.

Complete O-deacetylation of the product oligosides was carried out with sodium methoxide in methanol to give p-nitrophenyl N,N'-diacetyl-β-chitobioside (**9**; 68% yield), N,N',N"-triacetyl-β-chitotrioside (**10**; 76% yield), N,N',N",N"' -tetraacetyl-β-chitotetraoside (**11**; 65% yield), and N,N',N",N"',-N""-pentaacetyl-β-chitopentaoside (**12**; 77% yield), respectively. It should be noted here that the deacetylation of **7** and **8** was incomplete when performed at room temperature[5] giving an inseparable mixture of the partially acetylated oligosides based on high performance liquid chromatography. (Fig. 1a). Complete deacetylation was achieved on heating at reflux to give a sharp single HPLC peak (Fig. 1b).

Structures of all the products obtained here were assigned by elemental analyses and spectroscopic methods, except for **9** and **10** which were identified by comparison with the corresponding authentic samples.[4,5]

The overall yields of **9** (17.1%; ref.[3] 12.4%), **10** (23.5 %; ref.[4] 7%), **11** (17.6%; ref.[5] 1.4%, impure?), and **12** (15.5%) obtained here are significant from the standpoint of chemical synthesis. Compound **12** has recently been shown to be an excellent substrate for the assay of lysozyme in human urine as well as in hen egg-white, 20 times more sensitive toward the enzyme than is **11**.[8]

EXPERIMENTAL

General methods. Melting points were determined with a Yanaco MP-500 Micro-melting-point apparatus, and are uncorrected. High performance liquid chromatography (HPLC) was conducted with a Waters Model 560 apparatus having a column of silica gel [YMC-pack A 014 (30 cm x 6 mm)], the solvent composition being 1:3 water - acetonitrile; flow rate 1.0 mL/min; detection by U.V. at 215 nm and 300 nm with a Waters Lambda-Max Model 481 LC Spectrophotometer. Specific rotation was determined with a Digital Automatic Polarimeter PM-101 apparatus (Union Giken Corp., Ltd.). U.V.

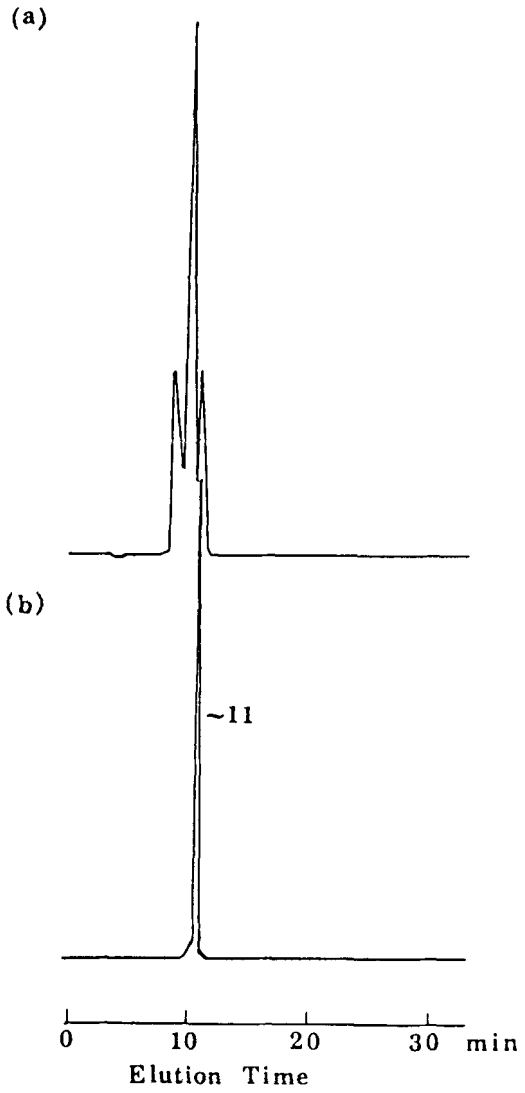

Fig. 1. HPLC analysis of a Mixture Obtained by O-Deacetylation of 7 with Sodium Methoxide in Methanol.

a) The reaction was performed according to the conditions described in reference 5, i.e., leaving mixture for 20 min at room temperature after the addition of NaOMe to a warm (40 C) methanolic solution of 7, and b) at reflux temperautre for 9 h. The elution pattern was obtained by monitoring at an absorbance of 300 nm.

spectra were taken on a Shimadzu Double Beam Spectrophotometer UV-200S apparatus. I.R. spectra were recorded on a JASCO Diffraction Grating Infra-red Spectrophotometer A-102 apparatus using KBr pellets. NMR spectra were recorded on a Varian XL-300 (for ^{1}H), a JEOL JNM FX-200 (for ^{13}C), a JEOL JNM GX-270 (for solid-phase), and GX-500 (for ^{13}C) apparatus. Elemental analyses were performed using a Perki-Elmer 240C apparatus at the Laboratory of Microanalysis, Shizuoka College of Pharmacy, Shizuoka, Japan.

Hydrochlorides of Chitobiose, Chitotriose, Chitotetraose, and Chitopentaose: These materials were prepared according to the method reported by Distler and Roseman.[6]

Preparation of Chitooligosaccharide Acetates

Chitobiose Octaacetate (**1**): To acetic anhydride (90 mL) containing fused zinc chloride (15 g, 110 mmol) was added portionwise chitobiose hydrochloride (15 g, 36.3 mmol) in 20 min with vigorous stirring, and the resulting suspension was further stirred for 20 min (complete dissolution) in an oil-bath (50 - 55 °C). After cooling to room temperature, the solution was poured onto ice-water (400 mL), and the resultant solution was neutralized with sodium bicarbonate (140 g). The mixture was extracted with chloroform (150 mL x 3), the organic layer was washed with chilled water (50 mL x 3), and dried over anhydrous sodium sulfate. After filtering off the desiccant, the organic solution was concentrated to a syrup, to which was added diethyl ether (200 mL) with trituration. After leaving the mixture overnight in a refrigerator, crystals were gathered by filtration, and dried *in vacuo* to give **1** (19 g, 77% yield): $[\alpha]_D^{20}$ +30.8°(c 0.52, DMSO); IR ν_{max} 3300 (NH), 1740 (OAc), 1650 (Amide I), and 1540 cm^{-1}(Amide II); ^{1}H NMR (300 MHz, DMSO-d_6 - TMS) δ 1.74 and 1.79 (6H, s x 2, N- and N'COC$\underline{H}_3$), 1.90, 1.91, 1.94, 1.95, 1.97, 1.99, 2.01, 2.02, 2.05, 2.06, and 2.16 (18H, OAc x 6), 3.53 - 3.64 (1H, m, H-2'), 3.70 - 4.04 (5.56H, m, H-2 , 4, 5, 5', 6b, and 6'b), 4.15 (0.48H, ddd, J = 11.5 Hz, 9.1 Hz, and 3.7 Hz, H-2), 4.25 - 4.37 (2H, m, H-6a and 6'a), 4.64 - 4.69 (1H, m, H-1'), 4.82 (1H, m, H-4'), 5.01 - 5.10 (1H, m, H-3), 5.13 (1H, t, $J_{2',3'} = J_{3',4'}$ = 9.5 Hz, H-3'), 5.62 (0.54H, d, $J_{1\beta,2}$ = 8.7 Hz, H-1β), 5.83 (0.46H, d, $J_{1\alpha,2}$ = 3.7 Hz, H-1α), and 7.88 - 8.03 (2H, m, N- and N'$\underline{H}$). The anomeric ratio

α:β = 46:54 was calculated from appropriate signal integrations.

Anal. Calcd for $C_{28}H_{40}N_2O_{17}$: C, 49.70; H, 5.96; N, 4.14. Found: C, 49.77; H, 6.00; N, 4.12.

Chitotriose Hendecaacetate (2): To acetic anhydride (120 mL) containing fused zinc chloride (20 g, 146.8 mmol) was added portionwise chitotriose hydrochloride (20 g, 32.7 mmol) in 30 min with vigorous stirring. The reaction temperature was kept at 50 - 55°C and stirred until a clear solution was obtained. The resulting solution was then worked up as above to give **2** (28.04 g, 87% yield): $[\alpha]_D^{20}$ +23.1° (c 0.52, DMSO); IR ν_{max} 3300 (NH), 1740 (OAc), 1660 (Amide I), and 1540 cm^{-1} (Amide II): ^1H NMR (DMSO-d_6 - TMS) δ 1.73, 1.74, and 1.79 (9H, s x 3, $NCOCH_3$ x 3), 1.90, 1.92, 1.94, 1.95, 2.00, 2.02, 2.04, 2.05, 2.08, 2.09, 2.15 (24H, OAc x 8), 3.43 - 4.17 (11H, m, H-2, 2', 2", 4, 4', 5, 5', 5", 6b, 6'b, and 6"b), 4.24 - 4.37 (3H, m, H-6a, 6'a, and 6"a), 4.54 - 4.59 (1H, m, H-1'), 4.64 (1H, d, $J_{1",2"}$ = 8.1 Hz, H-1"), 4.81 (1H, t, $J_{4",5"}$ = 9.5 Hz, H-4"), 4.97 - 5.07 (2H, m, H-3 and 3'), 5.12 (1H, t, $J_{2",3"} = J_{3",4"}$ = 9.5 Hz, H-3"), 5.60 (0.62H, d, $J_{1\beta,2}$ = 8.7 Hz, H-1β), 5.82 (0.38H, d, $J_{1\alpha,2}$ = 3.0 Hz, H-1α), and 7.87 - 7.99 (3H, m, NH, NH', and NH"). The anomeric ratio of α:β = 38:62 was calculated from appropriate signal integrations.

Anal. Calcd for $C_{40}H_{57}N_3H_{24}\cdot H_2O$: C, 48.93; H, 6.06; N, 4.28. Found: C, 48.90; H, 5.81; N, 4.26.

Chitotetraose Tetradecaacetate (**3**): Chitotetraose hydrochloride (20 g, 24.7 mmol) was added portionwise during 30 min with vigorous stirring to acetic anhydride (240 mL), in which was dissolved fused-zinc chloride (30 g, 220 mmol). The mixture was stirred at 55 - 60°C until a clear solution was obtained. The resulting mixture was worked up as descibed above to give **3** (29.5 g, 93% yield): $[\alpha]_D^{20}$ +16.6° (c 0.66, DMSO); IR ν_{max} 3300 (NH), 1735 (OAc), 1655 (Amido I), and 1530 cm^{-1}; ^1H NMR (DMSO-d_6 - TMS) δ 1.73, 1.74, and 1.78 (12H, s x 3, N-, N'-, N"-, and N"'-$COCH_3$), 1.90, 1.91, 1.94, 1.95, 2.00, 2.01, 2.03, 2.05, 2.08, and 2.15 (30H, OAc x 10), 3.43 - 4.16 (15H, m, H-2, 2', 2", 2"', 4, 4', 4", 5, 5', 5", 5"', 6b, 6'b, 6"b, and 6"'b), 4.24 - 4.37 (4H, m, H-6a, 6'a, 6"a, and 6"'a), 4.53 - 4.57 (2H, m, H-1' and 1"), 4.63 (1H, d,

$J_{1''',2'''}$ = 8.4 Hz, H-1'''), 4.81 (1H, t, $J_{4''',5'''}$ = 10.0 Hz, H-4'''), 4.93 - 5.07 (3H, m, H-3, 3', and 3"), 5.12 (1H, t, $J_{2,3}$ = $J_{3''',4'''}$ = 10.0 Hz, H-3'''), 5.6 (0.5H, d, $J_{1\beta,2}$ = 8.7 Hz, H-1β), 5.81 (0.5H, d, $J_{1\alpha,2}$ = 3.3 Hz, H-1α), and 7.87 - 7.98 (4H, m, NH x 4). The anomeric ratio of α:β = 1:1 was calculated from appropriate signal integrations.

Anal. Calcd for $C_{52}H_{74}N_4O_{31}\cdot 1.5H_2O$: C, 48.86; H, 6.07; N, 4.38. Found: C, 48.78; H, 5.84; N, 4.45.

Chitopentaose Heptadecaacetate (4): To acetic anhydride 240 mL) containing fused zinc chloride (30 g) was added portionwise chitopentaose hydrochloride (20 g, 19.9 mmol) was added in 30 min with vigorous stirring. The mixture was stirred for 40 min at 55 - 60°C. After the addition, an almost clear solution was obtained, but an insoluble mass precipitated during the course of heating. The resulting mixture was worked up as above described to give 4 (27 g, 86% yield); $[\alpha]_D^{20}$ +15.3°(c 0.59, DMSO); IR ν_{max} 3400 (NH), 1730 (OAc), 1655 (Amide I), and 1540 cm^{-1} (Amide II); ^1H NMR (DMSO-d_6 - TMS) δ 1.72, 1.74, 1.78, 1.80 (15H, s x 4, N-, N'-, N"-, N'''-, and N''''-$COCH_3$), 1.89, 1.89, 1.91, 1.94, 1.95, 2.00, 2.01, 2.03, 2.03, 2.04, 2.07, 2.07, 2.15 (36H, OAc x 12), 3.51 - 4.16 (19H, m, H-2, 2', 2", 2''', 2'''', 4, 4', 4", 4''', 5, 5', 5", 5''', 5'''', 6b, 6'b, 6"b, 6'''b, and 6''''b), 4.23 - 4.37 (5H, m, H-6a, 6'a, 6"a, 6'''a, and 6''''a), 4.52 - 4.57 (3H, m, H-1', 1", and 1'''), 4.63 (1H, d, $J_{1'''',2''''}$ = 7.8 Hz, H-1''''), 4.81 (1H, t, J = 9.8 Hz, H-4''''), 4.92 - 5.07 (4H, m, H-3, 3', 3", and 3'''), 5.12 (1H, t, J = 9.8 Hz, H-3''''), 5.59 (0.48H, d, $J_{1\beta,2}$ = 9.3 Hz, H-1β), 5.81 (0.52H, d, $J_{1\alpha,2}$ = 2.7 Hz, H-1α), and 7.87 - 8.01 (5H, m, NH x 5). The anomeric ratio of α:β = 52:48 as calculated from appropriate signal integrations.

Anal. Calcd for $C_{64}H_{91}N_5O_{38}\cdot 2H_2O$: C, 48.82; H, 6.08; N, 4.45. Found: C, 48.87; H, 5.93; N, 4.39.

Autocatalytic Fusion Reactions of p-Nitrophenol with Chitooligosaccharides Acetates

The Reaction with Chitobiose Heptaacetate (1): To p-nitrophenol (18.2 g, 130.8 mmol) fused in an oil-bath heated at 180 - 190°C, was added 1 (10 g, 14.8 mmol) while stirring with a glass rod. The resulting melt was allowed to react under diminished pressure (aspirator) for 15 min. The mixture

was cooled, and triturated with ethanol (40 mL) and allowed to stand in a refrigerator overnight. The resultant crystals were gathered by filtration, and washed with chilled ethanol, to give crude crystals of p-nitrophenyl heptaacetylchitobioside (5)(3.59 g, 32% yield), mp 248 - 250°C (dec). The crystals (3.50 g) were dissolved in methanol (500 mL) with heating, and the resulting solution was treated with activated charcoal (5 g). After filtering off the charcoal, the filtrate was concentrated to a volume of ca. 50 mL. Ethanol (250 mL) was added to the resultant suspension, which was then allowed to stand overnight in a refrigerator. The crystals were gathered by filtration and dried in vacuo to give 5 (2.67 g, 24% yield): mp 256 - 257°C (dec) [lit.[3] mp 257 - 258°C (dec)]; $[\alpha]_D^{20}$ -5.8° (c 0.53, DMSO){lit.[3] $[\alpha]_D^{23}$ -43°(c 0.21, chloroform)}; IR ν_{max} 3270 (NH), 1740 (OAc), 1655 (Amide I), 1540 (Amide II), 1590, 1490 (Ph), 1340 ($PhNO_2$), 860 (C-N of $PhNO_2$), and 840 (CH of Ph); ^1H NMR (DMSO-d_6 - TMS) δ 1.76, 1.78 (6H, s x 2, N- and N'-$COCH_3$), 1.91, 1.96, 1.99, 2.02, 2.04 (15H, s x 5, OAc x 5), 3.62 (1H, q, $J_{1',2'} = J_{2',3'} = J_{2',NH}$ = 9.3 Hz, H-2'), 3.81 - 4.12 (6H, m, H-2, 4, 5, 5', 6b, and 6'b), 4.29 (1H, dd, $J_{5,6a}$ 3.9 Hz and $J_{6a,6b}$ 12.7 Hz, H-6a), 4.39 (1H, d, $J_{6'a,6'b}$ = 10.7 Hz, H-6'a), 4.67 (1H, d, $J_{1',2'}$ = 9.3 Hz, H-1'), 4.84 (1H, t, $J_{4',5'}$ 9.3 Hz, H-4'), 5.09 (1H, t, $J_{2,3} = J_{3,4}$ = 9.6 Hz, H-3), 5.12 (1H, t, $J_{2',3'} = J_{3',4'}$ = 9.3 Hz, H-3'), 5.43 (1H, d, $J_{1,2}$ = 9.3 Hz, H-1), 7.19 (2H, d, o-phenyl proton x 2), 8.01 - 8.05 (2H, m, NH x 2), and 8.21 (2H, d, m-phenyl proton x 2), ^{13}C NMR (DMSO-d_6 - TMS) δ 20.2, 20.3, 20.5 (CH_3 of OAc), 22.6 (CH_3 of N- and N'Ac), 53.0 (C-2), 53.6 (C-2), 61.6 (C-6), 62.0 (C-6'), 68.2 (C-4'), 70.5 (C-3), 72.1 (C-3'), 72.3 (C-5), 72.9 (C-5'), 75.4 (C-4), 96.8 (C-1), 100.1 (C-1'), 116.4 (o-Ph carbon x 2), 125.4 (m-Ph carbon x 2), 142.0 (Ph carbon attached to the phenolic oxygen), 161.3 (p-Ph carbon), 168.9, 169.1, 169.2, 169.3, and 169.6 (C=O carbon of Ac x 7).

Anal. Calcd for $C_{32}H_{41}N_3O_{18}$: C, 50.86; H, 5.47; N, 5.56. Found: C, 50.69; H, 5.45: N, 5.52.

The Reaction with Chitotriose Hendecaacetate (2): To fused p-nitrophenol (9.0 g, 64.7 mmol) at 180 - 190°C, was added portionwise 2 (5.0 g, 5.1 mmol) while stirring with a glass rod. The resulting melt was kept at the same temper-

ature under diminished pressure (aspirator) for 15 min. The cooled mixture was worked up with methanol (25 mL) in the same way as described above to give crude crystals of **6** (2.01 g, 37% yield), mp 275 - 277°C (dec). The crystals (2.0 g) were then dissolved in 1:1 chloroform - methanol (200 mL) with heating, and the solution was treated with activated charcoal (5 g). After filtering off the charcoal, diethyl ether (40 mL) was added to the filtrate, and the mixture was left in a refrigerator overnight to give pure **6** (1.7 g, 32% yield): mp 280 - 282°C (dec) [lit.[4] m.p. 274 - 276°C (dec)]; $[\alpha]_D^{20}$ -15.4° (c 0.52, DMSO) {lit.[4] $[\alpha]_D^{26}$ -36.1° (c 0.36, pyridine)}; IR ν_{max} 3300 (NH), 1740 (OAc), 1660 (Amide I), 1520 (Amide II), 1590 (C=C of Ph), 1345 ($PhNO_2$), and 860 cm^{-1} (C-N of $PhNO_2$); ^1H NMR (DMSO-d_6 - TMS) δ 1.74, 1.75 (9H, N-, N'-, and N"-$COCH_3$), 1.90, 1.94, 1.95, 2.00, 2.03, 2.10 (21H, O-$COCH_3$ x 9), 3.47 - 4.12 (11H, m, H-2, 2', 2", 4, 4', 5, 5', 5", 6b, 6'b, and 6"b), 4.25 - 4.29 (2H, m, H-6a and 6'a), 4.39 (1H, d, $J_{6"a,6"b}$ = 10.7 Hz, H-6"a), 4.57 (1H, d, $J_{1',2'}$ = 8.3 Hz, H-1'), 4.65 (1H, d, $J_{1",2"}$ = 9.0 Hz, H-1"), 4.81 (1H, t, $J_{4",5"}$ = 9.8 Hz, H-4"), 4.96 - 5.05 (2H, m, H-3 and 3'), 5.13 (1H, t, $J_{2",3"} = J_{3",4"}$ = 9.8 Hz, H-3"), 5.41 (1H, d, $J_{1,2}$ = 8.2 Hz, H-1), 7.18 (2H, d, o-Ph proton x 2), 7.93 - 8.02 (3H, m, NH x 3), and 8.21 (2H, d, J = 9.3 Hz, m-Ph proton x 2); ^{13}C NMR (DMSO-d_6 - TMS) 20.6, 20.3 (CH_3 of OAc), 22.6 (CH_3 of NAc), 53.0 (C-2), 53.7 (C-2' and 2"), 61.6 (C-6), 62.0 (C-6'), 62.5 (C-6"), 68.2 (C-4"), 70.4 (C-3), 71.6 (C-3'), 72.2 (C-5 and 3"), 72.7 (C-5'), 72.8 (C-5"), 75.1 (C-4), 75.5 (C-4'), 96.8 (C-1), 99.9 (C-1'), 100.0 (C-1"), 116.4 (o-Ph carbon x 2), 125.4 (m-Ph carbon x 2), 142.0 (Ph carbon attached to the phenolic oxygen), 161.3 (p-Ph carbon), 168.9, 169.0, 169.1, 169.3, 169.6, 169.7, and 169.9 (C=O of Ac x 10).

Anal. Calcd for $C_{44}H_{58}N_4O_{25}$: C, 50.67; H, 5.61; N, 5.37. Found: C, 50.80; H, 5.63; N, 5.30.

The Reaction with Chitotetraose Tetradecaacetate (**3**): To fused p-nitrophenol (9.0 g, 64.7 mmol) was added **3** (5.0 g, 3.91 mmol) while stirring with a glass rod. The resulting melt was allowed to react at 180 - 190°C for 15 min under diminished pressure (aspirator). The mixture was cooled, triturated with methanol (25 mL) and left in a refrigerator

overnight. The crystals obtained were gathered by filtration, washed with methanol, and dried in vacuo to give 7 (2.3 g, 44% yield); mp 263 - 264°C (dec). The crytals (2.25 g) were dissolved in 1:1 chloroform - methanol (300 mL), and decolorized with activated charcoal (10 g). The filtrate was mixed with diethyl ether (80 mL) and left in a refrigerator overnight. The crystals obtained were gathered by filtration, washed with chilled 1:1 chloroform - methanol, and dried in vacuo to give pure 7 (1.44 g, 27% yield): mp 258 - 260°C (dec)[lit.[5] mp 266 - 267°C (dec)]; $[\alpha]_D^{20}$ -10.4°(c 0.48, DMSO); IR ν_{max} 3350 (NH), 1730 (OAc), 1655 (Amide I), 1530 (Amide II), 1590 (C=C, Ph), 1340 ($PhNO_2$), 860 (C-N of $PhNO_2$), and 845 cm^{-1} (C-H of Ph); ^1H NMR (DMSO-d_6 - TMS) δ 1.73, 1.74, 1.75, 1.75 (12H, s x 4, N-, N'-, N"-, and N‴-Ac), 1.90, 1.94, 1.95, 2.00, 2.03, 2.09 (27H, OCOCH_3 x 9), 3.43 - 4.11 (15H, m, H-2, 2', 2", 4, 4', 4", 5, 5', 5", 6b, 6'b, 6"b, and 6‴b), 4.24 - 4.29 (3H, m, H-6a, 6'a, and 6"a), 4.38 (1H, d, $J_{6'''a,6'''b}$ = 8.3 Hz, H-6‴a), 4.53 - 4.57 (2H, m, H-1' and 1"), 4.63 (1H, d, $J_{1''',2'''}$ = 8.3 Hz, H-1‴), 4.81 (1H, t, $J_{4''',5'''}$ = 9.8 Hz, H-4‴), 4.93 - 5.05 (3H, m, H-3, 3', and 3"), 5.12 (1H, t, $J_{2''',3'''} = J_{3''',4'''}$ = 9.8 Hz, H-3‴), 5.41 (1H, d, $J_{1,2}$ = 8.3 Hz, H-1), 7.18 (2H, d, o-Ph proton x 2), 7.89 - 8.02 (4H, m, NH x 4), and 8.21 (2H, d, J = 9.9 Hz, m-Ph proton x 2); ^{13}C NMR (DMSO-d_6 - TMS) δ 20.3. 20.6 (CH_3 of OAc), 22.5 (CH_3 of NAc), 53.0 (C-2), 53.7 (C-2', 2", and 2‴), 61.5 (C-6), 62.0 (C-6'), 62.5 (C-6" and 6‴), 68.2 (C-4‴), 70.4 (C-3), 71.6, 72.2 (C-5, 3', 3", and 3‴), 72.7 (C-5'), 73.0 (C-5"), 73.1 (C-5‴), 75.1, 75.5 (C-4, 4', and 4"), 96.8 (C-1), 99.7, 100.0 (C-1', 1", and 1‴), 116.4 (o-Ph carbon x 2), 125.4 (m-Ph carbon x 2), 142.0 (Ph carbon attached to the phenolic oxygen), 161.3 (p-Ph carbon), 168.8, 169.0, 169.0, 169.3, 169.6, 169.7, 169.8 (C=O of Ac x 13).

Anal. Calcd for $C_{56}H_{75}N_5O_{32}\cdot H_2O$: C, 49.89; H, 5.76; N, 5.19. Found: C, 49.76; H, 5.64; N, 5.15.

The Reaction with Chitopentaose Heptadecaacetate (4): To fused p-nitrophenol (18.5 g, 133 mmol) was added 4 (10 g, 6.35 mmol) while stirring with a glass rod. The resulting melt was allowed to react at 180 - 190°C for 15 min under diminished pressure (aspirator). The reaction mixture was cooled, triturated with methanol (40 mL), and left in a re-

frigerator overnight. The crystals obtained were gathered by filtration, washed with methanol, and dried in vacuo to give crude **8** (4.16 g). The crystals (4.15 g) were dissolved in 1:1 chloroform - methanol (1000 mL) with heating, the resulting solution was treated with activated charcoal (15 g), and, after filtering off the charcoal, the filtrate was mixed with diethyl ether (100 mL). After leaving the mixture in a refrigerator, the resultant crystals were gathered by filtration, washed with 1:1 chloroform - diethyl ether, and dried in vacuo to give **8** (2.15 g, 21% yield): mp 268 - 270°C (dec); $[\alpha]_D^{20}$ -2.0 (c 0.50, DMSO); IR ν_{max} 3400 (NH), 1735 (OAc), 1655 (Amide I), 1535 (Amide II), 1590 (C=C of Ph), 1345 ($PhNO_2$), 860 (C-N of $PhNO_2$), and 845 (C-H of Ph); ^1H NMR (DMSO-d_6 - TMS) δ 1.72, 1.73, 1.74, 1.75 (15H, $NCOCH_3$ x 5), 1.89, 1.93, 1.94, 2.00, 2.02, 2.07, 2.07, 2.08 (33H, $OCOCH_3$ x 11), 3.46 - 4.09 (19H, m, H-2, 2', 2", 2‴, 2⁗, 4, 4', 4", 4‴, 5, 5', 5", 5‴, 5⁗, 6b, 6'b, 6"b, 6‴b, and 6⁗b), 4.20 - 4.30 (4H, m, H-6a, 6'a, 6"a, and 6‴a), 4.38 (1H, d, $J_{6''''a,6''''b}$ = 10.9 Hz, H-6⁗a), 4.50 - 4.60 (3H, m, H-1', 1", and 1‴), 4.63 (1H, d, $J_{1'''',2''''}$ = 8.3 Hz, H-1⁗), 4.80 (1H, t, $J_{4'''',5''''}$ = 10.3 Hz, H-4⁗), 4.92 - 5.04 (4H, m, H-3, 3', 3", and 3‴), 5.12 (1H, t, $J_{2'''',3''''} = J_{3'''',4''''}$ = 10.3 Hz, H-3⁗), 5.41 (1H, d, $J_{1,2}$ = 8.3 Hz, H-1), 7.18 (2H, d, o-Ph proton x 2), 7.87 - 8.02 (5H, m, NH x 5), and 8.21 (2H, d, J = 8.3 Hz, m-Ph proton x 2); ^{13}C NMR (DMSO-d_6 - TMS) δ 20.6, 22.3 (CH_3 of OAc), 22.5 (CH_3 of NAc), 52.9 (C-2), 53.8, 53.9 (C-2', 2", 2‴, and 2⁗), 61.6 (C-6), 62.0 (C-6'), 62.4 (C-6", 6‴, and 6⁗), 68.1 (C-4⁗), 70.4 (C-3), 71.6, 72.2, 72.7, 73.0 (C-3', 3", 3‴, 3⁗, 5, 5', 5", 5‴, and 5⁗), 75.1, 75.5 (C-4, 4', 4", and 4‴), 96.8 (C-1), 99.7, 100.0 (C-1', 1", 1‴, and 1⁗), 116.4 (carbon of o-Ph x 2), 125.4 (carbon of m-Ph x 2), 141.9 (Ph carbon attached to the phenolic oxygen), 161.3 (carbon of p-Ph), 168.9, 169.0, 169.0, 169.6, and 169.7 (C=O x 16).

Anal. Calcd for $C_{68}H_{92}N_6O_{39}\cdot H_2O$: C, 49.94; H, 5.79; N, 5.14. Found: C, 49.88; H, 5.72; N, 5.11.

O-Deacetylation of p-Nitrophenyl Chitooligosaccharides Acetates

The Reaction of **5**: To a suspension of **5** (1.76 g, 2.33 mmol) in 1:1 chloroform - methanol (90 mL), was added 1.0 M sodium methoxide in methanol (0.5 mL) and the resulting mix-

ture was continued to stir for 1 h at room temperature. The suspension became a clear solution, and then again a suspension. The suspension was left in a refrigerator overnight, the resulting crystals were gathered by filtration, washed with chilled methanol, and dried *in vacuo* to give crude crystals of **9** (1.02 g, 81% yield), mp 220 - 223°C (dec). The crystals (1.02 g) were dissolved in water (100 mL), and the solution was treated with activated charcoal. After filtering off the charcoal, the filtrate was concentrated to a volume of 10 mL, to which was added ethanol (150 mL) and the mixture was left in a refrigerator overnight. The crystals precipitated were gathered by filtration, and dried *in vacuo* to give pure **9** (0.86 g, 71% yield): mp 228 - 230°C (dec) [*lit.*[3] mp 226 - 227°C (dec)]; $[\alpha]_D^{20}$ +1.96° (*c* 0.51, DMSO), UV λ_{max} 300 nm (ε 10000); IR ν_{max} 3500 - 3200 (OH, NH), 1640 (Amide I), 1540 (Amide II), 1590, 1490 (C=C of Ph), 1510, 1345 ($PhNO_2$), 860 (C-N of $PhNO_2$), and 845 (C-H of Ph); ^1H NMR (DMSO-d_6 - TMS) δ 1.83 (3H, s, $NCOCH_3$), 1.87 (3H, s, $N'COCH_3$), 3.02 - 3.84 (m, ring protons), 4.36 (1H, d, $J_{1',2'}$ 8.2 Hz, H-1'), 4.69 - 4.84 (3H, m, HO x 3), 5.00 - 5.11 (2H, m, HO x 2), 5.22 (1H, d, $J_{1,2}$ = 8.3 Hz, H-1), 7.18 (2H, d, *o*-Ph proton x 2), 7.76 (1H, d, $J_{NH,2}$ = 8.3 Hz, NH), 7.91 (1H, d, $J_{N'H,2'}$ = 9.3 Hz, N'H), 8.19 (2H, d, $J_{2,3}$ = 9.3 Hz, *m*-Ph proton x 2); ^{13}C NMR (DMSO-d_6 - TMS): δ 24.5 (N- and N'-$COCH_3$), 56.2 (C-2), 57.1 (C-2'), 61.3 (C-6), 62.7 (C-6'), 72.2 (C-4'), 73.8 (C-3), 75.6 (C-3'), 76.7 (C-5), 78.4 (C-5'), 82.0 (C-4), 99.7 (C-1), 103.5 (C-1'), 118.4 (*o*-Ph carbon x 2), 127.6 (*m*-Ph carbon x 2), 143.7 (Ph carbon attached to the phenolic oxygen), 163.7 (*p*-Ph carbon), and 172.6 (C=O of N- and N'-Ac).

Anal. Calcd for $C_{32}H_{31}N_3O_{13}\cdot 1.5H_2O$: C, 46.15; H, 5.98; N, 7.34. Found: C, 46.48; H, 5.54; N, 7.39.

The Reaction of **6**: To a solution of **6** (1.20 g, 1.15 mmol) in 1:1 chloroform - methanol (200 mL), was added 1.0 M sodium methoxide in methanol (2.0 mL), and the solution was stirred at room temperature for 1 h. After leaving the mixture in a refrigerator overnight, the resulting crystals were gathered by filtration and dried *in vacuo* to give crude crystals of **10** (0.85 g, 98% yield); mp 238 - 241°C (dec). The crude crystals (0.85 g) were dissolved in water (100 mL), and the

solution was treated with activated charcoal. After filtering off the charcoal, the filtrate was concentrated to a volume of 10 mL, to which was added methanol (200 mL) and the mixture was left in a refrigerator overnight. The crystals obtained were washed with methnol and dried *in vacuo* to give pure **10** (0.66 g, 74% yield): mp 242 - 244°C (dec) [*lit.*[4] mp 238 - 240°C (dec)]; $[\alpha]_D^{20}$ +9.7° (*c* 0.31, DMSO); UV λ_{max} 300 nm (ε 10000); IR ν_{max} 3450 - 3250 (OH, NH), 1650 (Amide I), 1545 (Amide II), 1590, 1495 (C=C of Ph), 1510, 1345 ($PhNO_2$), 860 (C-N of $PhNO_2$), and 845 (C-H of Ph); ^1H NMR (DMSO-d_6 - TMS) δ 1.84 (3H, s, $NCOCH_3$), 1.88 (6H, s, N'- and N"-$COCH_3$), 3.00 - 3.83 (m, ring protons), 4.34 (1H, d, $J_{1",2"}$ = 8.2 Hz, H-1"), 4.39 (1H, d, $J_{1',2'}$ = 8.3 Hz, H-1'), 4.74 - 4.81 (5H, m, HO x 5), 5.00 - 5.11 (2H, m, OH x 2), 5.23 (1H, d, $J_{1,2}$ = 9.0 Hz, H-1), 7.18 (2H, d, *o*-Ph proton x 2), 7.77 (1H, d, $J_{NH,2}$ = 9.3 Hz, NH), 7.86 (1H, d, $J_{N'H,2'}$ = 8.8 Hz, N'H), 7.92 (1H, d, $J_{N"H,2"}$ = 8.8 Hz, N"H), and 8.19 (2H, d, J = 9.3 Hz, *m*-Ph proton x 2); ^{13}C NMR (DMSO-d_6 - TMS) δ 24.5 (N-, N'-, and N"-$COCH_3$), 56.1 (C-2 and 2'), 57.1 (C-2"), 61.1 (C-6), 61.7 (C-6'), 62.6 (C-6"), 72.3 (C-4"), 74.0 (C-3 and 3'), 75.5 (C-3"), 76.4 (C-5), 76.8 (C-5'), 78.6 (C-5"), 81.9 (C-4), 83.2 (C-4'), 99.6 (C-1), 103.4 (C-1'), 103.6 (C-1"), 118.2 (*o*-Ph carbon x 2), 127.3 (*m*-Ph carbon x 2), 143.5 (Ph carbon attached to the phenolic oxygen), 163.7 (*p*-Ph carbon), 170.6 (C=O of NAc), and 170.8 (C=O of N'- and N"-Ac).

Anal. Calcd for $C_{30}H_{44}N_4O_8 \cdot 1.5H_2O$: C, 46.45; H, 6.11; N, 7.22. Found: C, 46.37; H, 5.74; N, 7.18.

The Reaction of **7**: To a suspension of **7** (0.52 g, 0.39 mmol) in methanol (100 mL), was added 1.0 M sodium methoxide in methanol (10 mL), and the solution was heated at reflux for 9 h. The resultant suspension was allowed to cool at room temperature overnight, and the crystals thus obtained were gathered by filtration. The crystals were dried *in vacuo* to give crude **11** (0.36 g, 97% yield), which was then subjected to purification by dissolution in cold water (160 mL), followed by treatment with activated charcoal, concentration of the solution to a volume of 10 mL, dilution with methanol (100 mL), and leaving the solution in a refrigerator overnight. Pure **11** (0.24 g, 64% yield) was obtained: mp 200 -

315°C (dec); $[\alpha]_D^{20}$ +2.4° (c 0.41, DMSO); UV λ_{max} 300 nm (ε 10000); IR ν_{max} 3500 - 3150 (OH, NH), 1640 (Amide I), 1540 (Amide II), 1590 (C=C of Ph), 1345 ($PhNO_2$), 860 (C-N of $PhNO_2$), 845 (C-H of Ph); ^{1}H NMR (DMSO-d_6 - TMS) δ 1.80 (3H, s, $NCOCH_3$), 1.82 (9H, s, N'-, N"-, and N"'-$COCH_3$), 3.00 - 3.80 (m, ring protons), 4.32 - 4.41 (3H, m, H-1', 1", and 1"'), 4.66 - 4.82 (7H, m, HO x 7), 4.99 - 5.10 (2H, m, HO x 2), 5.23 (1H, d, $J_{1,2}$ = 7.8 Hz, H-1), 7.18 (2H, d, o-Ph proton x 2), 7.75 (1H, d, $J_{NH,2}$ = 8.8 Hz, NH), 7.84 - 7.87 (2H, m, N'H and N"H), 7.92 (1H, d, $J_{N"'H,2"'}$ = 8.8 Hz, N"'H), 8.19 (2H, d, J = 8.3 Hz, m-Ph proton x 2); ^{13}C NMR (DMSO-d_6 - TMS) δ 24.5 (N-, N'-, N"-, and N"'-$COCH_3$), 56.1 (C-2, 2', and 2"), 57.0 (C-2"'), 61.7 (C-6, 6', and 6"), 62.6 (C-6"'), 72.2 (C-4"'), 74.0 (C-3, 3', and 3"), 75.5 (C-3"'), 76.3 (C-5, 5', and 5"), 78.5 (C-5"'), 82.9 (C-4), 83.1 (C-4' and 4"), 99.6 (C-1), 103.6 (C-1', 1", and 1"'), 118.2 (o-Ph proton x 2), 127.3 (m-Ph proton x 2), 143.3 (Ph carbon attached to the phenolic oxygen), 163.6 (p-Ph carbon), 170.7 (C=O of N- and N'-Ac), 170.9 (C=O of N"- and N"'-Ac).

Anal. Calcd for $C_{38}H_{57}N_5O_{23}\cdot 1.5H_2O$: C, 46.62; H, 6.18; N, 7.15. Found: C, 46.75; H, 6.20; N, 7.18.

The Reaction of **8**: To a suspension of **8** (0.75 g, 0.46 mmol) in methanol (150 mL), was added 1.0 M sodium methoxide in methanol (30 mL), and the suspension was heated at reflux for 14 h. On cooling the suspension, crystals precipitated out, and were gathered by filtration, and dried *in vacuo* to give crude **12** (0.53 g, 99% yield). The crystals (0.53 g) were dissolved in water (2000 mL), and the solution was treated with activated charcoal. After filtering off the charcoal, the filtrate was concentrated to a volume of 50 mL (suspension), to which was added methanol (200 mL). After leaving the mixture in a refrigerator overnight, the crystals were gathered by filtration, and washed with chilled methanol to give pure **12** (0.41 g, 75% yield): mp 205 - 315°C (dec); UV λ_{max} 300 nm (ε 10000); IR ν_{max} 3425 (OH), 3150 (NH), 1635 (Amide I), 1590 (Amide II), 1350 ($PhNO_2$), 860 (C-N of $PhNO_2$), and 850 cm^{-1} (C-H of Ph); ^{13}C NMR (CPMAS; adamantane as external standard, δ 29.5) δ 23.58 (CH_3 of N-, N'-, N"-, N"'-, and N""-Ac), 56.21 (C-2, 2', 2", 2"', and 2""), 62.04 (C-6, 6',

6", 6‴, and 6⁗), 71.36 (C-4⁗), 74.47 (C-3, 3', 3", 3‴, and 3⁗), 76.80 (C-5, 5', 5", 5‴, and 5⁗), 84.18 (C-4, 4', 4", and 4‴), 102.41 (C-1 attached to p-nitrophenoxy function), 105.16 (C-1', 1", 1‴, and 1⁗), 118.1 (o-Ph carbon x 2), 126.24 (m-Ph carbon x 2), 142.60 (Ph-carbon attached to the phenolic oxygen, 162.05 (p-Ph carbon), and 178.21 (C=O of NAc group x 5). It was impossible to dtermine the solution ^{1}H NMR spectrum of **12** due to its extremely low solubility.

Anal. Calcd for $C_{46}H_{70}N_6O_{28}\cdot 2H_2O$: C, 46.38; H, 6.26; N, 7.06. Found: C, 46.33; H, 6.13; N, 6.97.

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