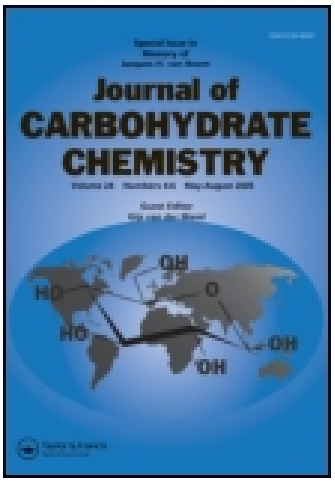

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Synthesis of α-D-Glucopyranosyl-(1-3)-α-D-Mannopyranosyl-(1-7)-4-Methylumbelliferone, A Fluorogenic Substrate for Endo-α-1,2-Mannosidase

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SYNTHESIS OF α-D-GLUCOPYRANOSYL-(1-3)-α-D-MANNOPYRANOSYL-(1-7)-4-METHYLUMBELLIFERONE, A FLUOROGENIC SUBSTRATE FOR ENDO-α-1,2-MANNOSIDASE

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ABSTRACT

α-D-Glucopyranosyl-(1-3)-α-D-mannopyranosyl-(1-7)-4-methylumbelliferone (Glc-Man-Muf) was synthesized as a potential fluorogenic substrate for endo-α-1,2-mannosidase. The synthesis was designed in a convergent way. The glucose donor ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-glucopyranoside and the mannose acceptor 1,2:4,6-di-*O*-isopropylidene-ß-D-mannopyranose were coupled in the presence of *N*-iodosuccinimide and trifluoromethanesulfonic acid to yield the corresponding disaccharide derivative. After conversion into peracetylated α-D-glucopyranosyl-(1-3)-α-D-mannopyranose the disaccharide was attached to 4-methylumbelliferone using the Helferich method. After separation of the desired isomer, deacetylation yielded the title compound. Glc-Man-Muf was used as a substrate in endomannosidase assays with rat liver Golgi preparations as an enzyme source (in the presence of the α-glucosidase inhibitor deoxynojirimycin). The degradation of Glc-Man-Muf was linear with protein up to 300 μg and with time up to 2 h. V_{max} and K_m were determined to be 0.17 nmol/mg x h and 3.7 mM, respectively.

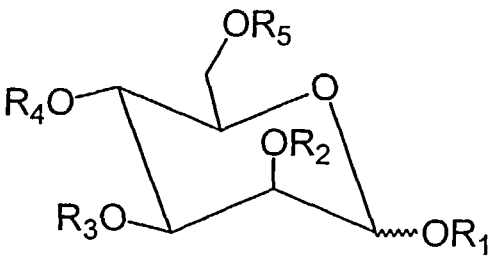
INTRODUCTION

Endo-α-1,2-mannosidase is involved in the processing of *N*-linked oligosaccharides. The enzyme cleaves $Glc_{1-3}Man$ from previously glucosylated oligosaccharides, thereby

providing a potential bypass on the way to complex *N*-linked carbohydrates, e.g., in glucosidase deficient cells.[1,2]

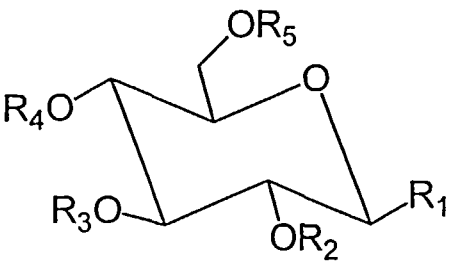
Determination of endomannosidase activities *in vitro* has been performed using [^{14}C]Glc_{1-3}-$Man_9GlcNAc_{1-2}$ oligosaccharides as substrates that were prepared either by metabolic labelling or by incubating microsome preparations with radioactively labelled sugar nucleotides (e.g., UDP-[^{14}C]Glc).[1,2] These substrates have three major disadvantages (if the use of radioactivity is not considered as a disadvantage):

- first, the preparation of the labelled oligosaccharides is rather complicated and time-consuming,
- second, only "radioactive" amounts can be obtained, i.e., an exact quantification of the material obtained is impossible,



	R_1	R_2	R_3	R_4	R_5
(1)	Ac	Ac	Ac	Ac	Ac
(2)	Br (-O)	Ac	Ac	Ac	Ac
(3)	=C(CH_3)-OMe		Ac	Ac	Ac
(4)	=C(CH_3)-OMe		H	H	H
(5)	=C(CH_3)-OMe		Bn	Bn	Bn
(6)	Ac	Ac	Bn	Ac	Ac
(7)	H	H	Bn	H	H
(8)	=CMe_2		Bn	=CMe_2	
(9)	=CMe_2		H	=CMe_2	

Scheme 1



	R_1	R_2	R_3	R_4	R_5
(10)	OAc	Ac	Ac	Ac	Ac
(11)	SEt	Ac	Ac	Ac	Ac
(12)	SEt	H	H	H	H
(13)	SEt	Bn	Bn	Bn	Bn

Scheme 2

third, the specific radioactivity of the oligosaccharides varies from preparation to preparation, i.e., the results obtained from different "batches" are not comparable.

Consequently endomannosidase units had to be defined as "the amount of enzyme that releases 1,000 dpm per h" as no absolute figures for enzyme activities and kinetic constants (V_{max} and K_m) could be obtained.

For a more convenient non-radioactive assay we decided to synthesize α-D-gluco-pyranosyl-(1-3)-α-D-mannopyranosyl-(1-7)-4-methylumbelliferone (Glc-Man-Muf) **20** as a potential fluorogenic substrate for endo-α-1,2-mannosidase.

RESULTS AND DISCUSSION

Synthesis of Glc-Man-Muf. The synthesis of Glc-Man-Muf was designed in a convergent way and the structure of every intermediate compound was verified by fast atom bombardment mass spectrometry and/or ^{1}H NMR. The glucose donor ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-glucopyranoside **13** (Scheme 2) and the mannose acceptor 1,2:4,6-di-*O*-isopropylidene-ß-D-mannopyranose **9** (Scheme 1) were produced separately and after

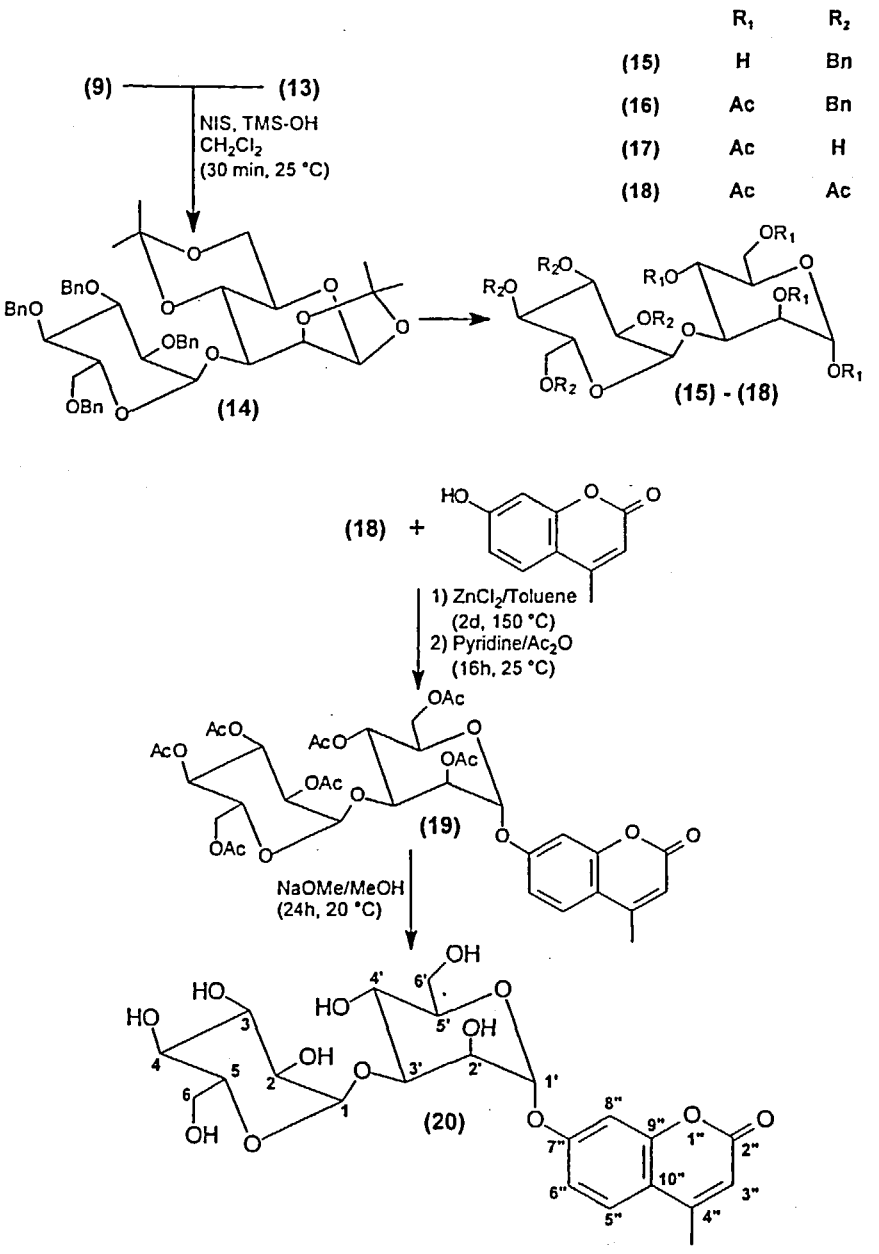
coupling the resulting disaccharide was converted to its octaacetyl derivative **18** (Scheme 3). The 4-methylumbelliferone was attached using the Helferich method and, after separation of the desired isomer, deacetylation yielded the title compound **20**.

Synthesis of 1,2:4,6-di-*O*-isopropylidene-ß-D-mannopyranose (9). The synthesis of a mannose derivative with a free OH group at position 3 was not trivial. A direct synthesis of **(9)** from mannose was not feasible. Using different acetonation procedures either 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose or 2,3:4,6-di-*O*-isopropylidene-D-mannopyranose were obtained.[3-6] Therefore, we had to prepare the 3-*O*-protected derivative **7** prior to acetonation.

Starting with peracetylated mannose **1**[7] the acetylated methyl orthoester **3** was prepared from the bromide **2**[8] in a yield of 95 %.[9] Via deacetylation (**4**, 99 %)[10] and benzylation the corresponding 3,4,6-tri-*O*-benzyl derivative **5** was obtained in 58 %.[11] Following was a sequential replacement of the 1,2-methoxyethylidene moiety and the benzyl residues by acetyl groups.[11] Compound **5** was reacted with a mixture of glacial acetic acid and acetic anhydride in the presence of sulfuric acid. The reaction conditions had to be optimized to achieve an excess of **6** over 1,2,6-tri-*O*-acetyl-3,4-di-*O*-benzyl-α-D-mannose and **1**. In our hands 2 h at 0 °C followed by 16 h at room temperature led to more than 80% of the desired compound **6**. Deacetylation (**7**, 68 %)[10] and subsequent reaction with 2,2-dimethoxypropane led to **8** (54 %)[5] from which **9** was obtained by catalytic hydrogenation in 32 % yield.[11]

Synthesis of ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (13). The synthesis of the glucose donor **13** was achieved in three steps starting from peracetylated β-D-glucose **10**. Compound **10** was thioethylated in the presence of $TiCl_4$ yielding **11** in a yield of 24 %.[12] Subsequent deacetylation (**12**, 100 %)[10] and benzylation led to **13** in 34 %.

Synthesis of α-D-glucopyranosyl-(1-3)-α-D-mannopyranosyl-(1-7)-4-methylumbelliferone (Glc-Man-Muf) (20). Compounds **9** and **13** were coupled to **14** by a reaction with *N*-iodosuccinimide in the presence of trifluoromethylsulfonic acid.[13] The ^{1}H NMR data (δ 4.98 (d, 1H, $J_{1,2}$=3.7 Hz, H-1, α-Glc, 82 %) and (δ 4.57 (d, 1H, $J_{1,2}$=7.5 Hz, H-1, β-Glc, 18 %)) showed that under the conditions used the reaction showed an anomeric specificity of more than 80 % in a total yield of 79 %. The 1,2,4,6,2',3',4',6'-octa-*O*-acetyl disaccharide **18** was obtained from **14** by cleavage of the isopropylidene moieties[14] (**15**, 100 %),



	R_1	R_2
(15)	H	Bn
(16)	Ac	Bn
(17)	Ac	H
(18)	Ac	Ac

Scheme 3

acetylation of the mannose residue (**16**, 100 %), debenzylation (catalytic hydrogenation, **17**, 59 %) and subsequent acetylation of the glucose (**18**, 56 %).

Separation of the β-glucosyl derivatives of **14**, **15**, **16**, and **17** could only be achieved with a strong decrease in yield. For example, purification of **16** (α-Glc) by chromatography on silica gel 60 (in a separate experiment) gave only 59 % product instead of 80 %. Therefore **15**, **16**, and **17** were used for the respective following reactions as crude products without further purification and only aliquots were purified for analysis. With the formation of **18**, the β-glucosyl derivative could be easily removed by recrystallization from methanol, and the isomers were obtained almost in the ratio predicted from the NMR data of **14** (see below).

Coupling of the disaccharide to 4-methylumbelliferone (Muf) turned out to be difficult. Neither the Koenigs-Knorr method via the heptaacetylated bromide nor the trichloroacetimidate procedure yielded considerable amounts of **19**. Therefore, we tried to attach **18** directly to Muf using the Helferich method.[15] The reaction was performed in a screw-capped vial. **18**, Muf, and $ZnCl_2$ were suspended in toluene and heated for 2 days at 150 °C. (If necessary additional $ZnCl_2$ was applied to the mixture and heating was prolonged for 2 days.) In order to eventually replace cleaved acetyl groups, the toluene was evaporated and the residue was treated with pyridine and acetic anhydride. Despite the rather extreme reaction conditions **19** was obtained in a yield of 75%. Deacetylation of **19** finally led to the title compound **20** in a yield of 75 %.

Glc-Man-Muf as Substrate for Endo-α-1,2-Mannosidase. Since endomannosidase has been reported to be Golgi-residing[2] we choose rat liver Golgi that is routinely prepared in our laboratory[e g 16] as an enzyme source. Golgi vesicles were incubated with **20** in the presence of Triton X-100 and the α-glucosidase inhibitor deoxynojirimycin as described in the EXPERIMENTAL section and the amount of Muf was determined by fluorescence measurement.

Endomannosidase activity is linear with the protein amount up to 300 μg (not shown) and with time up to 2 h (Fig. 1a). From the enzyme kinetics the apparent V_{max} and K_m values was determined to 0.17 nmol/(mg·h) and 3.7 mM, respectively, for the Golgi preparation used (Lineweaver-Burk plot see Fig. 1b). These results demonstrate that **20** is indeed a suitable fluorogenic substrate for the endo-α-1,2-mannosidase.

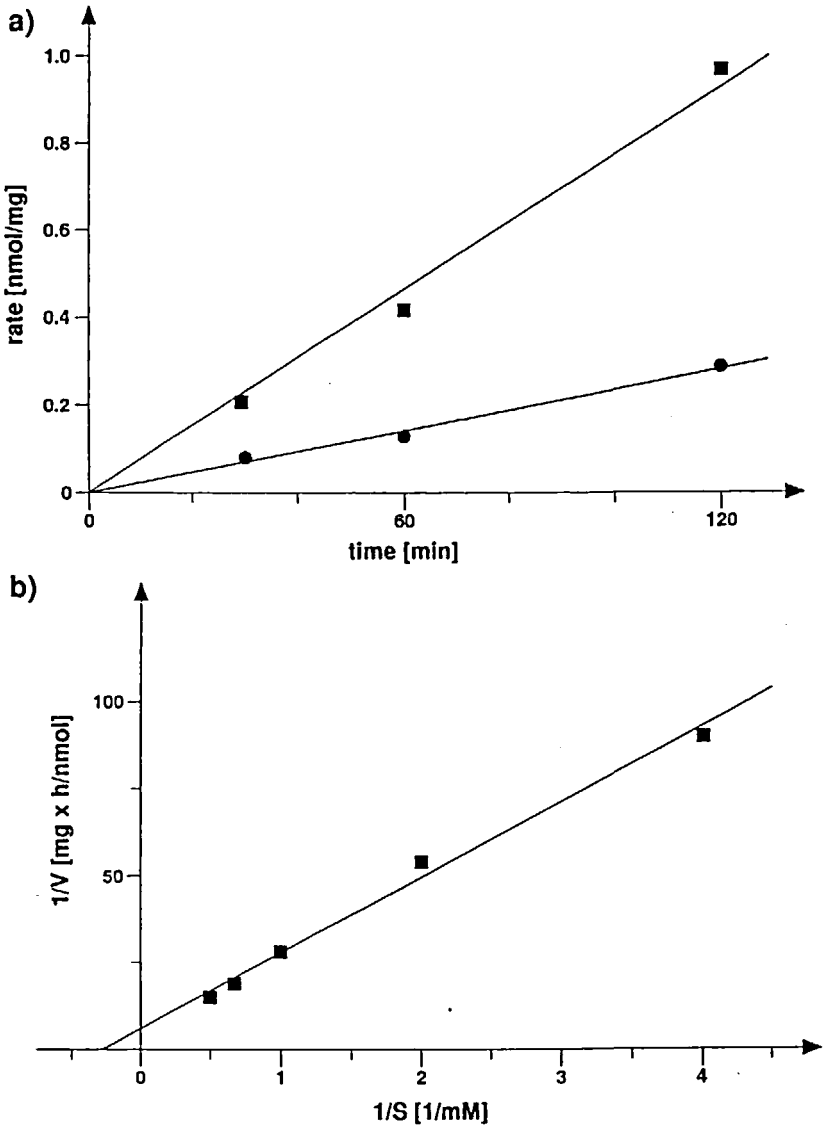


Figure 1. Time dependency (a)) and Lineweaver-Burk plot (b)) of endomannosidase activity in Golgi preparations from rat liver determined using Glc-Man-Muf as substrate.

a) Glc-Man-Muf (1 mM) was incubated with 100 μg (●) and 250 μg (■) Golgi protein for the indicated time periods as described in the text.

b) 0.25, 0.5, 1.0, 1,5, and 2.0 mM Muf was incubated with 250 μg Golgi protein for the 2 h as described in the text. The Lineweaver-Burk plot was calculated from the 1/V and 1/S values by linear regression ($K_r > 0.995$).

EXPERIMENTAL

General Methods. Melting points were determined on a Kofler-Weygand melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded at 500 MHz with a Bruker AMX-500 spectrometer. Thin-layer chromatography was performed on precoated plates of silica gel (Merck). Silica gel 60 (400-630 and 630-200 mesh, Merck) was used for column chromatography. All solvents and reagents were purified and dried before use.

FAB Mass Spectrometry. The FAB mass spectra were recorded on a VG analytical ZAB-HF reverse geometry mass spectrometer (V.G. Analytical, Manchester, U.K.). For atom bombardment xenon was used and the applied acceleration voltage was 7 kV. 1-Mercapto-2,3-propanediol (thioglycerol, Tgl) was used as matrix and 1 μL of a homogeneous sample solution containing 1 nmol/μL in chloroform/methanol (1:1, v/v) was added. The spectra were run in a mass range from 50 to 1200 atom mass units (amu) with a scan rate of 10 sec per decade and positive ions (FAB(+)) were detected. The spectra were recorded and evaluated on a SAM II/68 K computer (KWS, Ettlingen, FRG) using the DP10 program of AMD (Harpstedt, FRG).

2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl-(1-3)-1,2:4,6-di-*O*-isopropylidene-β-D-mannopyranose (14). 9 (1 g, 3.85 mmol), **13** (1.7 g, 2.93 mmol),. *N*-iodosuccinimide (1.7 g, 7.56 mmol) and 1.72 g 4 Å molecular sieves were dried for two days over P_2O_5. The mixture was suspended under an argon atmosphere in dry dichloromethane (45 mL) and trifluoromethylsulfonic acid (686 μL) dissolved in dry dichloromethane (52 mL) were dropped at 0 °C. After stirring for 30 min at 20 °C the suspension was neutralized with saturated sodium hydrogencarbonate and the iodine was removed by washing with aqueous sodium thiosulfate. The organic phase was concentrated *in vacuo*. Chromatography on silica gel 60 (light petroleum/ethyl acetate 4:1 (v/v)) yielded a syrup containing 80 % of **14** (1.78 g, 79 %; in relation to the glucosyl donor).[13] ^{1}H NMR ($CDCl_3$) δ 7.37-7.22 (m, 20H, aromat.), 5.32 (d, 1H, $J_{1,2}$=2.0 Hz, H-1'$_{(Glcα)}$), 5.27 (d, 1H, $J_{1,2}$=2.0 Hz, H-1'$_{(Glcβ)}$), 4.98 (d, 1H, $J_{1,2}$=3.7 Hz, H-1, α-Glc), 4.57 (d, 1H, $J_{1,2}$=7.5 Hz, H-1, β-Glc). The remaining signals could not be assigned. FAB MS: *m/z* 805 ($M+Na^+$), 181, 91.

2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl-(1-3)-α-D-mannopyranose (15). **14** (1.78 g, 2.3 mmol) was heated in 60% acetic acid (54 mL) for 2 h at 100 °C. After concentrating the solution *in vacuo* **15** was obtained as a syrup and was used without further

purification (1.61 g, 100 %).[14] FAB MS: *m/z* 725 ($M+Na^+$), 635, 523, 415, 325, 307, 181, 91.

2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl-(1-3)-1,2,4,6-tetra-*O*-acetyl-α-D-mannopyranose (16). **15** (1.61 g, 2.3 mmol) was dissolved in 8 mL of pyridine/acetic anhydride 1:1 (v/v) and the mixture was stirred for 16 h at 20 °C. The solution was concentrated *in vacuo* and residual pyridine was removed by azeotropic distillation with toluene. **16**, a syrup (2 g, 100 %), was immediately used for the next step. ^1H NMR ($CDCl_3$) δ 7.36-7.10 (m, 20H, aromat.), 6.04 (d, 1H, $J_{1,2}$=2.0 Hz, H-1'), 5.42 (dd, 1H, $J_{2,1}$=3.5 Hz, $J_{2,3}$=10.0 Hz, H-2), 5.28 (t≈dd, 1H, $J_{3,2}$=10.0 Hz, $J_{3,4}$=10.0 Hz, H-3*), 5.27 (t≈dd, 1H, $J_{4,3}$=10.0 Hz, $J_{4,5}$=10.0 Hz, H-4*), 5.15 (d, 1H, $J_{1,2}$=3.5 Hz, H-1), 4.98 (d, 1H, J=11.0 Hz, CH_2Ph), 4.88 (d, 1H, J=11.0 Hz, CH_2Ph), 4.87 (d, 1H, J=11.0 Hz, CH_2Ph), 4.83 (d, 1H, J=11.0 Hz, CH_2Ph), 4.80 (d, 1H, J=11.0 Hz, CH_2Ph), 4.79 (d, 1H, J=12.0 Hz, CH_2Ph), 4.64 (d, 1H, J=11.0 Hz, CH_2Ph), 4.59 (d, 1H, J=12.0 Hz, CH_2Ph), 4.44 (dd, 1H, $J_{6a,6b}$=12.0 Hz, $J_{6a,5}$=2.0 Hz, H-6a), 4.23 (ddd, 1H, $J_{5,4}$=10.0 Hz, $J_{5,6a}$=2.0 Hz, $J_{5,6b}$=5.0 Hz, H-5), 4.12 (dd, 1H, $J_{6b,6a}$=12.0 Hz, $J_{6b,5}$=5.0 Hz, H-6b), 2.01-2.05 (4 x s, 4 x 3H, CH_3-C=O). The remaining signals could not be assigned; *exchangeable. FAB MS: *m/z* 893 ($M+Na^+$), 811, 523, 415, 331, 307, 181, 169, 91.

α-D-Glucopyranosyl-(1-3)-1,2,4,6-tetra-*O*-acetyl-α-D-mannopyranose (17). **16** (2 g, 2.3 mmol) was dissolved in 120 mL methanol/ethyl acetate 2:1 (v/v) and 5 % palladium on charcoal (3 g) was added. The mixture was stirred under hydrogen for 16 h at 20 °C. After removal of the catalyst the solution was concentrated *in vacuo* and the residue was chromatographed on silica gel 60 (toluene/ethanol 4:1 (v/v)). **17** was obtained as syrup (580 mg, 49 %). Rechromatography of the product containing side fractions yielded an additional 121 mg of **17** (overall yield 59.8 %) FAB MS: *m/z* 533 ($M+Na^+$), 451, 371, 331, 289, 229, 169.

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl-(1-3)-1,2,4,6-tetra-*O*-acetyl-α-D-mannopyranose (18). **17** (580 mg, 1.14 mmol) was dissolved in 8 mL of pyridine/acetic anhydride 1:1 (v/v) and the mixture was stirred for 16 h at 20 °C. The solution was concentrated *in vacuo* and the crude product was purified by chromatography on silica gel 60 (toluene/acetone 10:1 (v/v)). **18** was recrystallized from methanol (431 mg, 55.8 %), mp 129 °C. (From the mother liquor 120.9 mg of the corresponding β-glucopyranosyl derivative **(18b)** could be isolated, over all yield 71.4%.) **18**: ^1H NMR ($CDCl_3$, COSY) δ 6.09 (d, 1H,

$J_{1,2}$=2.0 Hz, H-1'), 5.35 (t≈dd, 1H, $J_{4,3}$=10.0 Hz, $J_{4,5}$=10.0 Hz, H-4'), 5.34 (t≈dd, 1H, $J_{3,2}$=10.5 Hz, $J_{3,4}$=10.0 Hz, H-3), 5.29 (d, 1H, $J_{1,2}$=4.0 Hz, H-1), 5.24 (dd, 1H, $J_{2,1}$=2.0 Hz, $J_{2,3}$=4.0 Hz, H-2'), 5.04 (t≈dd, 1H, $J_{4,3}$=10.0 Hz, $J_{4,5}$=10.0 Hz, H-4), 4.76 (dd, 1H, $J_{2,1}$=4.0 Hz, $J_{2,3}$=10.5 Hz, H-2), 4.26 (dd, 1H, $J_{6a,6b}$=12.5 Hz, $J_{6a,5}$=4.5 Hz, H-6a'), 4.24 (dd, 1H, $J_{3,2}$=4.0 Hz, $J_{3,4}$=10.0 Hz, H-3'), 4.19 (dd, 1H, $J_{6a,6b}$=12.5 Hz, $J_{6a,5}$=5.0 Hz, H-6a), 4.12 (ddd, 1H, $J_{5,4}$=10.0 Hz, $J_{5,6a}$=4.5 Hz, $J_{5,6b}$=2.0 Hz, H-5'), 4.07 (dd, 1H, $J_{6b,6a}$=12.5 Hz, $J_{6b,5}$=2.0 Hz, H-6b'), 4.06 (dd, 1H, $J_{6b,6a}$=12.5 Hz, $J_{6b,5}$=2.5 Hz, H-6b), 3.91 (ddd, 1H, $J_{5,4}$=10.0 Hz, $J_{5,6a}$=5.0 Hz, $J_{5,6b}$=2.5 Hz, H-5), 2.20, 2.19, 2.18, 2.10, 2.09, 2.03, 2.02, 2.01 (each s, each 3H, CH_3-C=O). FAB MS: *m/z* 701 ($M+Na^+$), 619, 331, 169. **18b:** ^{1}H-NMR ($CDCl_3$, COSY): δ 6.05 (d, 1H, $J_{1,2}$=2.2 Hz, H-1'), 5.27 (t≈dd, 1H, $J_{4,3}$=9.8 Hz, $J_{4,5}$=10.0 Hz, H-4'), 5.19 (dd, 1H, $J_{2,1}$=2.2 Hz, $J_{2,3}$=3.4 Hz, H-2'), 5.17 (t≈dd, 1H, $J_{3,2}$=9.2 Hz, $J_{3,4}$=9.4 Hz, H-3), 5.09 (t≈dd, 1H, $J_{4,3}$=9.4 Hz, $J_{4,5}$=9.9 Hz, H-4), 4.87 (dd, 1H, $J_{2,1}$=7.7 Hz, $J_{2,3}$=9.2 Hz, H-2), 4.62 (d, 1H, $J_{1,2}$=7.7 Hz, H-1), 4.36 (dd, 1H, $J_{6a,6b}$=12.4 Hz, $J_{6a,5}$=4.1 Hz, H-6a), 4.24 (dd, 1H, $J_{6a,6b}$=12.4 Hz, $J_{6a,5}$=5.2 Hz, H-6a'), 4.13 (dd, 1H, $J_{3,2}$=3.4 Hz, $J_{3,4}$=9.8 Hz, H-3'), 4.11 (dd, 1H, $J_{6b,6a}$=12.4 Hz, $J_{6b,5}$=2.5 Hz, H-6b'), 4.09 (dd, 1H, $J_{6b,6a}$=12.4 Hz, $J_{6b,5}$=2.6 Hz, H-6b), 3.98 (ddd, 1H, $J_{5,4}$=10.0 Hz, $J_{5,6a}$=5.2 Hz, $J_{5,6b}$=2.5 Hz, H-5'), 3.73 (ddd, 1H, $J_{5,4}$=9.9 Hz, $J_{5,6a}$=4.1 Hz, $J_{5,6b}$=2.6 Hz, H-5), 2.17, 2.13, 2.09, 2.08, 2.06, 2.02, 2.01, 1.98 (each s, each 3H, CH_3-C=O). FAB MS: *m/z* 701 ($M+Na^+$), 619, 331, 169.

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl-(1-3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1-7)-4-methylumbelliferone (19). **18** (143.3 mg, 211.4 μmol) and 4-methylumbelliferone (148.7 mg, 844 μmol) were dried over P_2O_5 and suspended in 5 mL of dry toluene under argon in a screw-capped vial. Anhydrous zinc chloride (15 mg) was added and the mixture was heated for 2 days at 150 °C. After addition of more zinc chloride (15 mg) heating was prolonged for 2 more days. The solvent was evaporated under a stream of nitrogen and 20 mL of pyridine/acetic anhydride 1:1 (v/v) were added to the residue. The mixture was stirred for 16 h at 20 °C. The solvents were removed under a stream of nitrogen and the crude product was purified by chromatography on silica gel 60 (toluene/acetone 8:1 (v/v)) to give **19** as a syrup (130 mg, 75 %). ^{1}H NMR ($CDCl_3$, COSY) δ 7.53 (d, 1H, $J_{5,6}$=8.8 Hz, H-5"), 7.08 (d, 1H, $J_{8,6}$=2.4 Hz, H-8"), 6.99 (dd, 1H, $J_{6,5}$=8.8 Hz, $J_{6,8}$=2.4 Hz, H-6"), 6.20 (s≈d, 1H, $J_{3,CH3}$=1.3 Hz, H-3"), 5.59 (d, 1H, $J_{1,2}$=1.9 Hz, H-1'), 5.45 (dd, 1H, $J_{2,1}$=1.9 Hz, $J_{2,3}$=3.7 Hz, H-2'), 5.38 (t≈dd, 1H, $J_{4,3}$=9.9 Hz, $J_{4,5}$=10.1 Hz, H-4'), 5.37 (dd, 1H, $J_{3,2}$=10.2 Hz, $J_{3,4}$=9.6 Hz, H-3), 5.33 (d, 1H, $J_{1,2}$=3.9 Hz, H-1), 5.03 (t≈dd, 1H, $J_{4,3}$=9.6 Hz,

$J_{4,5}$=10.2 Hz, H-4), 4.78 (dd, 1H, $J_{2,1}$=3.9 Hz, $J_{2,3}$=10.3 Hz, H-2), 4.42 (dd, 1H, $J_{3,2}$=3.7 Hz, $J_{3,4}$=9.9 Hz, H-3'), 4.27 (dd, 1H, $J_{6a,6b}$=12.2 Hz, $J_{6a,5}$=5.0 Hz, H-6a), 4.18 (dd, 1H, $J_{6a,6b}$=12.2 Hz, $J_{6a,5}$=5.7 Hz, H-6a'), 4.17 (ddd, 1H, $J_{5,4}$=10.2 Hz, $J_{5,6a}$=5.0 Hz, $J_{5,6b}$=2.3 Hz, H-5), 4.11 (dd, 1H, $J_{6b,6a}$=12.2 Hz, $J_{6b,5}$=2.3 Hz, H-6b), 4.00 (dd, 1H, $J_{6b,6a}$=12.2 Hz, $J_{6b,5}$=2.5 Hz, H-6b'), 3.91 (ddd, 1H, $J_{5,4}$=10.1 Hz, $J_{5,6a}$=5.7 Hz, $J_{5,6b}$=2.5 Hz, H-5'), 2.41 (d, 3H, $J_{CH3,3}$=1.3 Hz, Muf-CH_3), 2.29, 2.09, 2.05, 2.02, 2.01, 2.04 (each s, each 3H, CH_3-C=O), 2.06 (s, 6H, 2 x CH_3-C=O).^{13}C NMR ($CDCl_3$) δ 170.67, 170.60, 170.58, 170.53, 169.85, 169.77, and 169.74 (CH_3-C=O) 160.76 (C-2"), 158.01, 154.97, 152.08, and 115.67 (C-4", C-7", C-9", C-10"), 125.91 (C-5"), 113.48 (C-3"), 113.33 (C-6"), 104.61 (C-8"), 96.77 (C-1), 95.91 (C-1'), 72.36 (C-3'), 70.90 (C-2), 70.21 (C-2'), 69.65 (C-5'), 69.22 (C-3), 68.44 (C-5), 68.23 (C-4), 67.87 (C-4'), 62.11 (C-6'), 62.04 (C-6), 20.93, 20.87, 20.78, 20.74, 20.71, and 20.69 (CH_3-C=O) 18.75 (Muf-CH_3). FAB MS: *m/z* 795 ($M+H^+$), 619, 331, 169.

α-D-Glucopyranosyl-(1-3)-α-D-mannopyranosyl-(1-7)-4-methylumbelliferone (20). **19** (100 mg, 125.9 μmol) was stirred in 10 mL 0.01 M solution of sodium methanolate in methanol for 18 h at 20 °C. The mixture was concentrated under a stream of nitrogen and the product was purified by chromatography on silica gel 60 (chloroform/ methanol/water 80:20:2 (v/v/v)). **20** was obtained as a syrup (47.5 mg, 75.6 %). ^{1}H NMR (D_2O/CD_3OD, COSY) δ 7.70 (d, 1H, $J_{5,6}$=8.5 Hz, H-5"), 7.13 (dd, 1H, $J_{6,5}$=8.5 Hz, $J_{6,8}$=2.2 Hz, H-6"), 7.12 (d, 1H, $J_{8,6}$=2.2 Hz, H-8"), 6.21 (s≈d, 1H, $J_{3,CH3}$=1.2 Hz, H-3"), 5.64 (d, 1H, $J_{1,2}$=2.0 Hz, H-1'), 5.26 (d, 1H, $J_{1,2}$=4.0 Hz, H-1), 4.33 (t≈dd, 1H, $J_{2,1}$=2.0 Hz, $J_{2,3}$=3.4 Hz, H-2'), 4.09 (dd, 1H, $J_{3,2}$=3.4 Hz, $J_{3,4}$=9.8 Hz, H-3'), 3.97 (t≈dd, 1H, $J_{4,3}$=9.8 Hz, $J_{4,5}$=9.8 Hz, H-4'), 3.87 (m, 2H, not resolved, H-5, H-6a*), 3.78 (dd, 1H, $J_{3,2}$=10.0 Hz, $J_{3,4}$=9.3 Hz, H-3), 3.72 - 3.77 (m, 3H, not resolved, H-6b*, H-6a'*, H-6b'*), 3.62 (dt≈ddd, 1H, $J_{5,4}$=9.8 Hz, $J_{5,6a}$=4.2 Hz, $J_{5,6b}$=4.2 Hz, H-5'), 3.54 (dd, 1H, $J_{2,1}$=4.0 Hz, $J_{2,3}$=10.0 Hz, H-2), 3.38 (t≈dd, 1H, $J_{4,3}$=9.3 Hz, $J_{4,5}$=9.7 Hz, H-4), 2.42 (s, 3H, Muf-CH_3); *exchangeable. ^{13}C NMR (D_2O/CD_3OD) δ 165.83 (C-2"), 160.89, 157.88, 156.08, and 116.84 (C-4", C-7", C-9", C-10"), 128.44 (C-5"), 116.15 (C-6"), 113.07 (C-3"), 105.64 (C-8"), 102.68 (C-1), 100.01 (C-1'), 80.77 (C-3'), 75.92 (C-5'), 75.10 (C-3), 74.50 (C-5), 74.06 (C-2), 71.85 (C-4), 71.55 (C-2'), 67.55 (C-4'), 62.77 and 62.57 (C-6, C-6'), 19.75 (Muf-CH_3). FAB MS: *m/z* 501 ($M+H^+$), 339, 177.

Assay for Endo-α-mannosidase. The endomannosidase assay contained in a total volume of 100 μL up to 2.5 mM **20**, 0.1% (w/v) Triton X-100, 100 mM phosphate buffer (pH 7), 2 mM deoxynojirimycin and up to 300 μg Golgi protein. The mixture was incubated

for up to 2 h at 37 °C and the reaction was stopped by adding 900 µL of a solution of 0.2 M sodium carbonate and 0.2 M glycine. The liberated Muf was quantified by determination of its fluorescence ($\lambda_{excitation}$= 365 nm, $\lambda_{emission}$= 455 nm).

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