An easy access to a 3,6-branched mannopentaoside bearing one terminal [1-^{13}C]-labeled D-mannopyranose residue*

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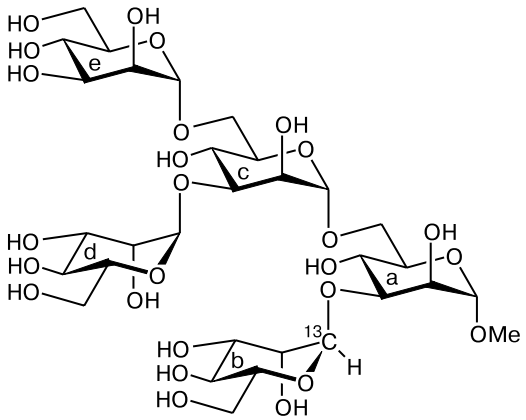
Methyl 2,4-di-*O*-benzoyl-α-D-mannopyranoside was used as a key intermediate in the synthesis of 3,6-branched mannopentaoside bearing one terminal D-[1-^{13}C]mannopyranose residue, *viz.*, methyl 6-*O*-[3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl)-3-*O*-{α-D-[1-^{13}C]mannopyranosyl}-α-D-mannopyranoside.

Key words: methyl 2,4-di-*O*-benzoyl-α-D-mannopyranoside, 3,6-branched mannooligosaccharides, D-[1-^{13}C]mannopyranose, [1-^{13}C]-labeled mannopentaoside, selective glycosylation, selective deacylation.

It is well known that carbohydrates play an important role in various recognition processes, for example, in intercellular interactions and immune reactions. Detailed investigation of binding of carbohydrates to protein receptors may be of considerable importance for the design of potential modulators of such interactions. The structures of specific carbohydrate-protein complexes can be studied by the site-directed replacement of amino acids of the receptor or modifications of monosaccharide residues of the ligand followed by calorimetry[1] or by X-ray diffraction.[2] NMR spectroscopy is a much more popular and accessible method, which is widely used for the determination of the conformations of carbohydrates in free state and after their binding to proteins.[3—5] This method is particularly useful in combination with enrichment of oligosaccharides with stable isotopes, for example, with the ^{13}C isotope.[6,7]

Mannose-binding proteins (MBP) are widespread in plants and animals. Mannose-specific mammalian lectins are involved in various immune processes, including innate immunity.[8—10] Recently,[11] antitumor activity of MBP has been revealed. Synthetic analogs of natural carbohydrate structures can be useful as ligands in elucidating the character of binding of the latter to receptors. In the present study, we synthesized one of such compounds, 3,6-branched mannopentaoside containing one terminal D-[1-^{13}C]mannopyranose residue, *viz.*, methyl 6-*O*-[3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl]-3-*O*-{α-D-[1-^{13}C]mannopyranosyl}-α-D-mannopyranoside (**1**-^{13}C).



1-^{13}C

Results and Discussion

Stepwise and block syntheses of the above-mentioned mannopentaoside with natural ^{13}C isotope abundance have been described repeatedly (see, for example, Refs 12—15). Our approach to the synthesis of selectively ^{13}C-labeled mannopentaoside is based on the use of readily accessible methyl 2,4-di-*O*-benzoyl-α-D-mannopyranoside (**2**) as a key intermediate and its successive transformation into a (1—6)-linked disaccharide by selective mannosylation, then into a 3,6-branched trisaccharide containing the D-[1-^{13}C]mannopyranose residue, and, finally, into a pentasaccharide through bis-mannosylation.

Compound **2** was prepared by a one-pot method (Scheme 1) analogous to that described earlier for the synthesis of octyl and tetradecyl α-D-mannopyranoside derivatives.[16] Treatment of methyl α-D-mannopyranoside

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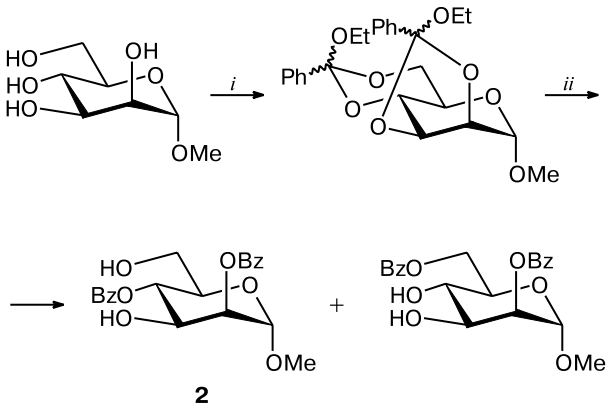
with triethyl orthobenzoate in the presence of TsOH and CF_3COOH in MeCN afforded 2,3:4,6-bis(ethyl orthobenzoate), which was subjected to acid hydrolysis without isolation. The target compound **2** and its isomer, *viz.*, methyl 2,6-di-*O*-benzoyl-α-D-mannopyranoside, which differ substantially in chromatographic mobilities, were isolated in 30 and 45% yields, respectively. Their ^{1}H NMR spectroscopic data are similar to those of analogous octyl and tetradecyl α-D-mannopyranoside derivatives.[16]

Scheme 1

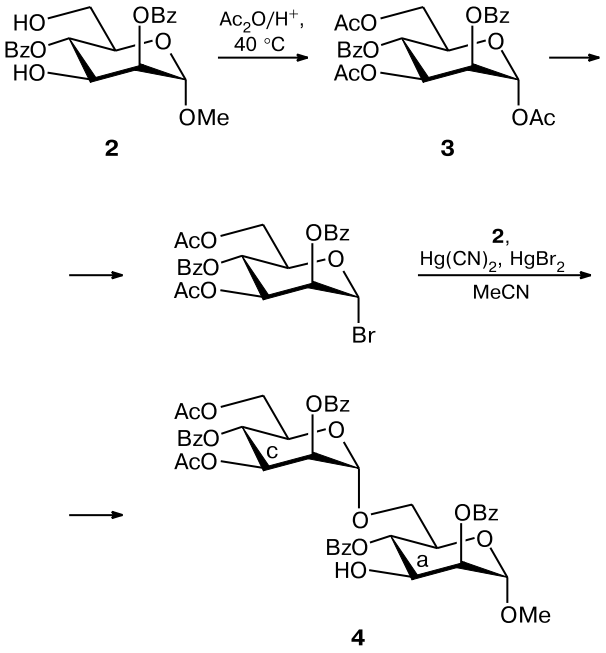


Reagents and conditions: *i.* $PhC(OEt)_3$, H^+; *ii.* H_3O^+.

In the synthesis of disaccharide, methyl 2,4-di-*O*-benzoyl-α-D-mannopyranoside (**2**) served as both the glycosyl acceptor and a precursor of a glycosyl donor. Acetolysis of glycoside **2** (Ac_2O—AcOH—H_2SO_4, 40 °C) afforded triacetate **3**, which was then transformed into the corresponding glycosyl bromide according to a standard procedure.[17] The Helferich condensation (MeCN, $Hg(CN)_2$ + $HgBr_2$) of this glycosyl donor with diol **2** occurs regioselectively to give (1—6)-linked disaccharide **4** in 57% yield (Scheme 2).

The presence of the 1—6 linkage in disaccharide **4** was established by ^{13}C NMR spectroscopy. The C(6a) atom resonates at substantially lower field (δ 66.49) than the C(6c) atom (δ 62.56) due to the α effect of glycosylation (see Ref. 18).* The presence of a free hydroxy group at the C(3a) atom in the derivative of disaccharide **4** provides the possibility to perform the site-directed introduction of the ^{13}C-labeled mannopyranose residue, while selective *O*-deacetylation[19] of the resulting 3,6-branched trisaccharide allows one to carry out bis-mannosylation at the O(3c) and O(6c) positions. It should be noted that we have used selective *O*-deacetylation of disaccharide **4** in

Scheme 2



the synthesis of an analog of mannopentaoside **1**-^{13}C containing three terminal nonreducing D-[1-^{13}C]mannopyranose residues.[20]

Before the synthesis of mannopentaoside **1**-^{13}C, we prepared its unlabeled analog. For this purpose, disaccharide **4** was initially transformed into trisaccharide **5** in 78% yield by the reaction with 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide in the presence of silver trifluoromethanesulfonate (AgOTf) (Scheme 3). The selective removal of the *O*-acetyl protective groups under the conditions of mild acidic methanolysis where the *O*-benzoyl groups remained intact[19] afforded diol **6** in a nearly quantitative yield.

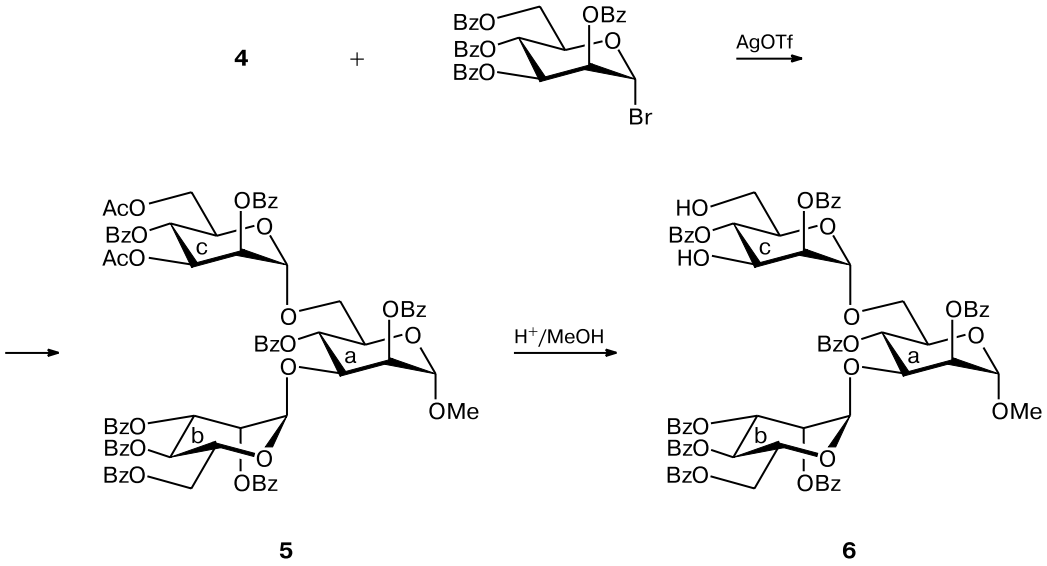
Diol **6** was used as the glycosyl acceptor in condensation with 1.8 equiv. of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide in a CH_2Cl_2—toluene mixture in the presence of AgOTf (Scheme 4). The yield of the bis-mannosylation product, *viz.*, fully protected mannopentaoside **7**, was ~70%.

The structures of compounds **5—7** were confirmed by NMR spectroscopy. In particular, the NMR spectrum of diacetate **5** shows signals of acetyl groups (δ_H 1.90 and 1.80, δ_C 20.59 and 20.48 (Me); 169.74 and 170.40 (CO)), which disappear in the spectrum of diol **6** In addition, the signals for H(6c) and H(6′c) in the spectrum of diol **6** are shifted upfield (δ_H 4.22 → 3.67 and 3.60), whereas the signals for C(2c), C(4c), and C(5c) are shifted downfield (δ_C 70.20 → 72.81, 66.99 → 70.27, and 68.78 → 70.72, respectively) (Tables 1 and 2).

The assignment of the signals for the protons and carbon atoms in the NMR spectra of perbenzoate **7** was

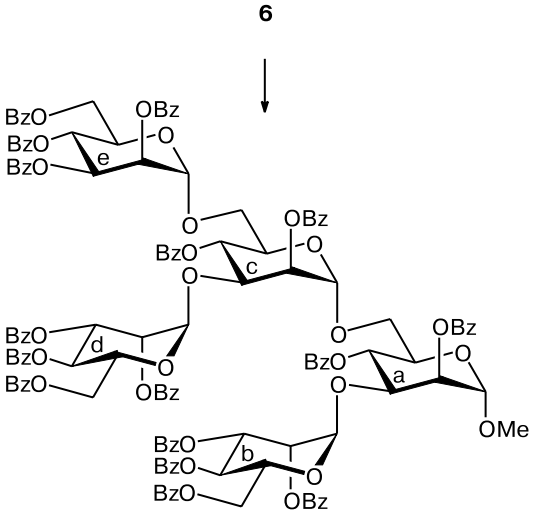
* The α-effects of glycosylation revealed in the cited study[18] refer to unprotected monosaccharide units. The signs of these effects remain unchanged for the *O*-acylated sugars, although they can differ in magnitude.

Scheme 3



5 **6**

Scheme 4



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made using 2D NMR techniques (COSY, ROESY, and HSQC) with account of the spectroscopic data for fully protected 3,6-branched mannooligosaccharides.[14] Of signals for the anomeric H and C atoms, those of the units involved in 1—3 linkages (b and d) are observed at the lowest field, whereas the signal of the terminal unit with the 1—6 linkage (e) appears at the highest field. The chemical shift of the anomeric proton of the latter unit (δ_H 4.83) is close to that observed earlier[20] (δ_H 4.87) for an analogous unit in the spectrum of a mannopentaoside containing three terminal nonreducing D-[1-^{13}C]mannopyranose residues.

Table 1. ^{1}H NMR spectroscopic data

Compound	Residue	δ						
		H(1)	H(2)	H(3)	H(4)	H(5)	H(6)	H(6′)
5	a	5.03	5.70—5.80	4.66	5.84	4.24	4.09	3.77
	b	5.36	5.37	5.70—5.80	6.00	4.55	4.63	4.41
	c	5.08	5.60	5.70—5.80	4.38	4.22		
6	a	5.03	5.71	4.61—4.64	5.96	4.18	4.04	3.77
	b	5.33	5.39	5.76	6.01	4.57	4.60	4.40
	c	5.14	5.52	4.61—4.64	5.54	4.00	3.67	3.60
7	a	5.02	5.71	4.66	6.04	4.34	4.24	3.84
	b	5.32	5.34	5.69	5.94	4.55	4.60	4.38
	c	5.23	5.82	4.73	6.00	4.33	4.05	3.50
	d	5.37	5.45	5.74	6.06	4.45	4.56	4.32
	e	4.83	5.63	5.94	6.09	4.43	4.50	4.30

Table 2. ^{13}C NMR spectroscopic data

Compound	Residue	δ					
		C(1)	C(2)	C(3)	C(4)	C(5)	C(6)
5	a	98.61	71.89	76.06	68.78	69.51	66.80
	b	99.55	70.13	69.34	66.65	69.64	62.74
	c	97.23	70.20	69.13	66.99	68.78	62.74
6	a	98.61	71.75	76.02	68.47	69.38	66.64
	b	99.40	70.27	69.38	66.64	69.63	62.70
	c	97.76	72.81	68.62	70.27	70.72	61.22
7	a	98.62	71.95	76.14	68.38	69.37	66.53
	b	99.50	70.13	69.57	66.61	69.49	62.72
	c	97.54	71.95	77.10	68.10	69.37	66.20
	d	99.94	70.13	69.65	66.29	69.49	62.53
	e	97.36	70.13	70.20	66.46	69.32	62.42

The Zemplén debenzoylation (MeONa in MeOH) of perbenzoate **7** afforded a free mannopentaoside having structure **1** with natural ^{13}C isotope abundance. The ^{13}C NMR spectrum of the latter was identical to the spectrum of the pentasaccharide synthesized earlier.[14]

The same sequence of reactions, including mannosylation of glycosyl acceptor **4**, *O*-deacetylation, and bismannosylation of the trisaccharide glycosyl acceptor, was used for the synthesis of the target mannopentaoside **1**-^{13}C.

Naturally, the first glycosylation was carried out with 2,3,4,6-tetra-*O*-benzoyl-α-D-[1-^{13}C]mannopyranosyl bromide prepared from the corresponding pentabenzoate by treatment with hydrogen bromide in AcOH. Condensation of this glycosyl donor with disaccharide derivative **4** in the presence of AgOTf under the conditions of the synthesis of unlabeled trisaccharide **5** afforded trisaccharide **5**-^{13}C in 69% yield.

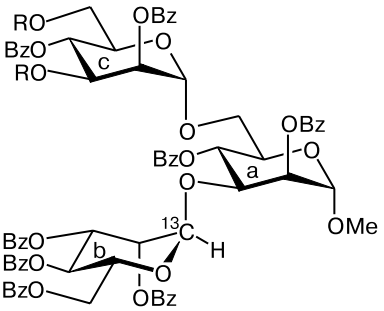
The chemical shifts of the anomeric H atoms in the ^1H NMR spectrum of the latter compound (δ 5.04, 5.37, and 5.09 for H(1a), H(1b), and H(1c), respectively) are virtually identical to those observed in the spectrum of the unlabeled analog (*cf.* Table 1). The signal for the anomeric H atom of the [1-^{13}C]-labeled mannopyranose residue, H(1b), appears as a doublet with $^1J_{H,C}$ = 172 Hz, which proves the α configuration of the resulting mannosidic bond. In other respects, the ^1H NMR spectrum of trisaccharide **5**-^{13}C is identical to that of **5**.

The ^{13}C NMR spectra of these trisaccharides are also similar to each other. The spectrum of compound **5**-^{13}C is characterized by high intensity of the signal for the anomeric C atom of the residue b (δ 99.61).

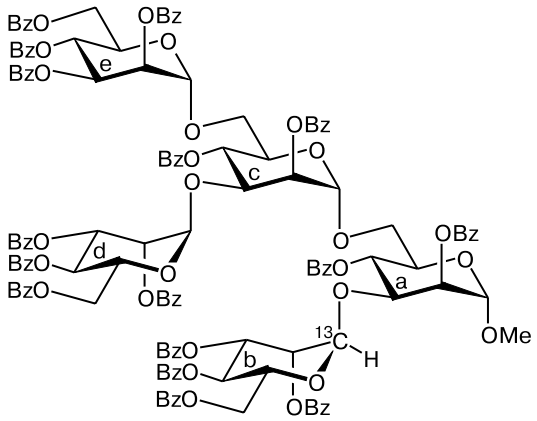
Deacetylation of diacetyl derivative **5**-^{13}C with HCl in MeOH gave rise to labeled trisaccharide diol **6**-^{13}C in 96% yield.

The latter compound was bis-mannosylated under the conditions described above for the synthesis of mannopentaoside **7**. The glycosylation product **7**-^{13}C was isolated in 68% yield. The ^1H NMR spectrum of this compound differed from the spectrum of the unlabeled perbenzoylated mannopentaoside only in that it had a doublet for H(1b) (δ 5.34, $^1J_{H,C}$ = 173 Hz). The corresponding chemical shifts of all other H atoms of both compounds are the same within 0.02 ppm. The ^{13}C NMR spectra of these compounds are also virtually identical (the differences in the chemical shifts are at most 0.1 ppm).

The target mannopentaoside **1**-^{13}C was prepared by debenzoylation of the corresponding perbenzoate, *viz.*, compound **7**-^{13}C. In our opinion, the structure of this pentasaccharide is convincingly supported by a comparison of the ^1H NMR spectrum of labeled mannopentaoside **1**-^{13}C with the spectrum of authentic mannopentaoside **1** (see Fig. 1). As can be seen from Fig. 1, the doublet for the anomeric proton H(1b) in the spectrum of mannopentaoside **1**-^{13}C (*a*) ($^1J_{H,C}$ = 172 Hz) is transformed into



5-^{13}C: R = Ac
6-^{13}C: R = H



7-^{13}C

a broadened singlet in the spectrum measured with the use of broad-band {^{13}C}-^1H heteronuclear decoupling (*b*). The latter spectrum is virtually identical to the spectrum (*c*) of the authentic sample of mannopentaoside **1** with natural ^{13}C isotope abundance.

To summarize, we synthesized the branched ^{13}C-labeled mannopentaoside starting from methyl 2,4-di-*O*-benzoyl-α-D-mannopyranoside as a key intermediate. This mannopentaoside can be used as a probe for NMR studies of interactions of mannose-binding proteins with ligands.

Experimental

The optical rotation was measured on a PU-07 digital polarimeter (Russia) in chloroform. The ^1H and ^{13}C NMR spectra were recorded on AC-200, WM-250, AM-300, and DRX-500 spectrometers (Bruker) in $CDCl_3$ with Me_4Si as the internal standard (the spectra of compound **1**-^{13}C were measured in D_2O with $(CH_3)_2CO$ as the internal standard). The assignment of the signals in the spectra was made using the COSY, ROESY, and HSQC techniques. The NMR spectroscopic data are given in Tables 1 and 2; the chemical shifts of the protons of the benzoyl residues and MeO groups, the multiplicities of the signals (in the tables), and the coupling constants characteristic of mannopyranosides are omitted.

Column chromatography was carried out on Silpearl silica gel (Czech Republic), and TLC analysis was performed on Merck

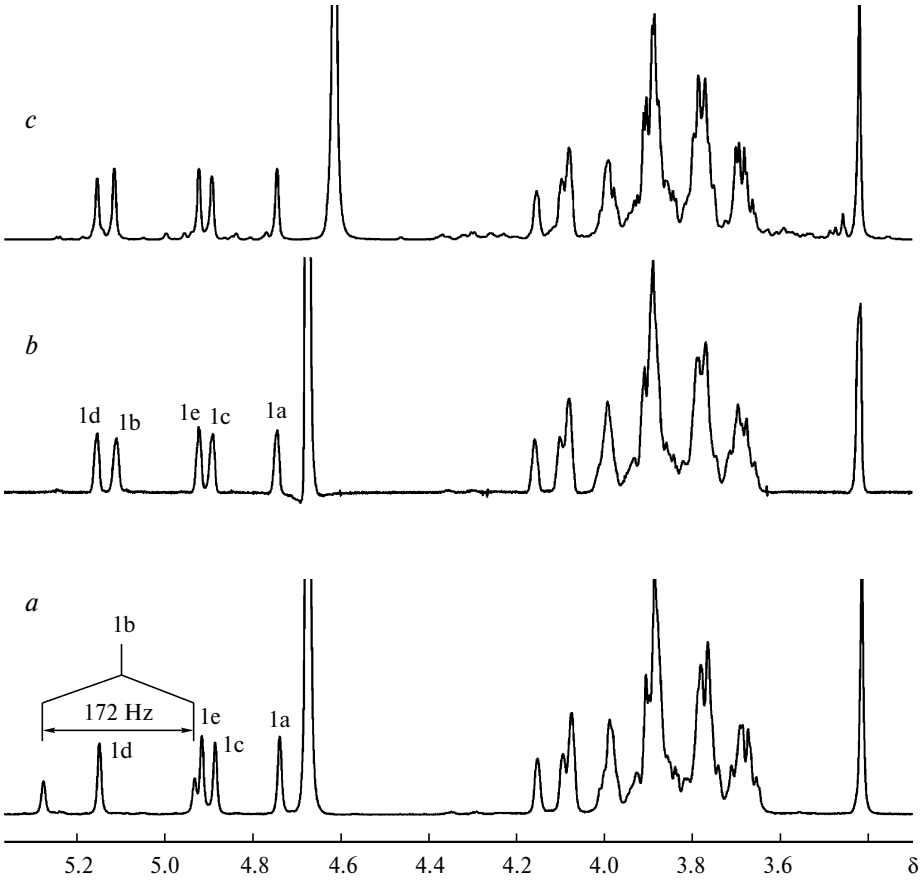


Fig. 1. ^1H NMR spectra (D_2O) of (*a*) mannopentaoside **1**-^{13}C, (*b*) of the same pentasaccharide after broad-band {^{13}C}-^1H heteronuclear decoupling, and (*c*) of a sample of mannopentaoside **1** with natural ^{13}C isotope abundance.[14] The notations 1a—1e refer to the signals for the anomeric H atoms of the residues a—e, respectively.

DC-Alufolien Kieselgel 60_{254} plates; toluene—ethyl acetate mixtures were used as eluents for column chromatography and solvents for TLC; visualization was performed with UV light and by spraying plates with dilute H_2SO_4 followed by heating at ~150 °C.

Toluene was distilled over Na. Dichloromethane and acetonitrile were distilled successively over P_2O_5 and CaH_2. Commercial methyl α-D-mannopyranoside (Sigma), triethyl orthobenzoate (Aldrich), and D-[1-^{13}C]mannose (Omicron Biochemicals, Inc.) were used.

2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl bromide and its [1-^{13}C]-labeled analog were synthesized in virtually quantitative yields by the reaction of hydrogen bromide (20 equiv., a 40% solution in AcOH, which was prepared from AcBr and the calculated amount of H_2O in AcOH) with solutions of the corresponding pentabenzoates in chloroform or dichloromethane for 2 days followed by standard work up.[17]

Methyl 2,4-di-*O*-benzoyl-α-D-mannopyranoside (2) (*cf.* lit. data[16]). Triethyl orthobenzoate (2.9 g, 2.9 mL, 13 mmol) and a solution of TsOH (60 mg) in MeCN (6.5 mL) and CF_3COOH (0.03 mL) were added with stirring to a suspension of methyl α-D-mannopyranoside (0.97 g, 5 mmol) in MeCN (80 mL). The reaction mixture was stirred at ~40 °C until complete dissolution was achieved (~1 h), and then the solvent was evaporated. The residue was dissolved in MeCN (45 mL), after which 90% CF_3COOH (4 mL) was added. The solution was kept at ~20 °C for 20 min and concentrated. Column chromatography of the residue afforded isomers, *viz.*, compound **2** and methyl 2,6-di-*O*-benzoyl-α-D-mannopyranoside.

Compound 2, the yield was 0.6 g (30%), $[\alpha]_D$ −35.4 (*c* 1.3). Found (%): C, 63.01; H, 5.87. $C_{21}H_{22}O_8$. Calculated (%): C, 62.68; H, 5.51. ^1H NMR, δ: 5.50 (t, 1 H, H(4)); 5.41 (dd, 1 H, H(2)); 4.91 (br.s, 1 H, H(1)); 4.41 (dd, 1 H, H(3)); 3.95 (m, 1 H, H(5)); 3.84 (dd, 1 H, H(6)); 3.75 (dd, 1 H, H(6′)). ^{13}C NMR, δ: 98.44 (C(1)); 72.68 (C(2)); 70.31 (C(4)); 70.17 (C(5)); 68.34 (C(3)); 61.32 (C(6)).

Methyl 2,6-di-*O*-benzoyl-α-D-mannopyranoside, the yield was 0.9 g (45%), $[\alpha]_D$ +11.8 (*c* 1.33). Found (%): C, 62.68; H, 5.71. ^1H NMR (200 MHz, *c* 0.5 g mL^{-1}), δ: 5.32 (dd, 1 H, H(2)); 4.79 (br.s, 1 H, H(1)); 4.15 (dd, 1 H, H(3)); 4.02 (t, 1 H, H(4)); 3.86 (m, 1 H, H(5)); 4.60 (m, 2 H, H(6), H(6′)). ^{13}C NMR, δ: 98.47 (C(1)); 72.29 (C(2)); 70.38 (C(5)); 69.76 (C(3)); 67.60 (C(4)); 63.48 (C(6)).

1,3,6-Tri-*O*-acetyl-2,4-di-*O*-benzoyl-D-mannopyranose (3). A cold solution of concentrated H_2SO_4 (0.1 mL) in Ac_2O (2.5 mL) was added to a solution of methyl glycoside **2** (0.34 g, 0.81 mmol) in AcOH (2.5 mL) and Ac_2O (2.5 mL) cooled to 0 °C. The mixture was heated at 40 °C for 2 h. After completion of the reaction (TLC control), the solution was cooled to ~20 °C, water was added dropwise, and the solution was concentrated *in vacuo*. The residue was dissolved in toluene and the solution

was concentrated. A solution of the residue in chloroform was washed with a $NaHCO_3$ solution. Acetate **3** was obtained in a yield of 0.41 g (98.5%), $[\alpha]_D$ −39.1 (*c* 2.55). Found (%): C, 61.21; H, 5.24. $C_{26}H_{26}O_{10}$. Calculated (%): C, 60.70; H, 5.09. ^{1}H NMR, δ: 6.30 (br.s, 1 H, H(1)); 5.80 (t, 1 H, H(4)); 5.68 (dd, 1 H, H(3)); 5.57 (dd, 1 H, H(2)); 4.40—4.20 (m, 3 H, H(5), H(6), H(6′)). ^{13}C NMR, δ: 90.63 (C(1)); 70.66 (C(5)); 68.94 (C(3)); 68.74 (C(2)); 66.43 (C(4)); 62.49 (C(6)).

Methyl 2,4-di-*O*-benzoyl-6-*O*-(3,6-di-*O*-acetyl-2,4-di-*O*-benzoyl-α-D-mannopyranosyl)-α-D-mannopyranoside (4). Acetic acid (1.5 mL), then AcBr (0.32 mL, 4.25 mmol, 5 equiv.), and aqueous AcOH (80 μL of H_2O in 1.6 mL of AcOH) were added to a solution of acetate **3** (0.44 g, 0.85 mmol) in chloroform (1.5 mL) cooled to 0 °C. The mixture was kept at ~20 °C for ~16 h and diluted with chloroform. The solution was washed with ice water, a $NaHCO_3$ solution cooled to 0 °C, and water, dried by filtration through a layer of cotton, and concentrated. 3,6-Di-*O*-acetyl-2,4-di-*O*-benzoyl-α-D-mannopyranosyl bromide was obtained in a yield of 0.42 g (92%), $[\alpha]_D$ −10.1 (*c* 1.09).

A solution of 3,6-diol **2** (0.24 g, 0.6 mmol), $Hg(CN)_2$ (152 mg, 0.6 mmol), and $HgBr_2$ (43 mg, 0.12 mmol) in MeCN (3 mL) was stirred with ground 3 Å molecular sieves for 1 h and then a solution of the glycosyl bromide obtained in MeCN (2 mL) was added. After 30 min (disappearance of the glycosyl bromide, TLC control), the reaction mixture was filtered and the filtrate was diluted with chloroform. The solution was washed with water and a KI solution and concentrated. The residue was chromatographed. Disaccharide derivative **4** was obtained in a yield of 293 mg (57%), $[\alpha]_D$ −19.3 (*c* 1.77). Found (%): C, 63.76; H, 5.32. $C_{45}H_{44}O_{17}$. Calculated (%): C, 63.08; H, 5.18. ^{1}H NMR, δ: 5.76 (dd, 1 H, H(3c)); 5.67 and 5.68 (both t, 1 H each, H(4a), H(4c)); 5.60 (dd, 1 H, H(2c)); 5.47 (dd, 1 H, H(2a)); 5.06 (br.s, 1 H, H(1c)); 4.98 (br.s, 1 H, H(1a)); 4.41 (dd, 1 H, H(3a)); 4.23 (m, 1 H, H(5a)); 4.17 (m, 1 H, H(5c)); 4.07 (dd, 1 H, H(6c)); 4.04 (dd, 1 H, H(6′c)); 4.01 (dd, 1 H, H(6a)); 3.74 (dd, 1 H, H(6′a)). ^{13}C NMR, δ: 98.70 (C(1a)); 97.32 (C(1c)); 72.88 (C(2a)); 70.15 (C(4a)); 70.06 (C(2c)); 69.15 (C(3a)); 69.11 (C(3c)); 69.00 (C(5a)); 68.78 (C(5c)); 66.81 (C(4c)); 66.49 (C(6a)); 62.56 (C(6c)).

Methyl 2,4-di-*O*-benzoyl-6-*O*-(3,6-di-*O*-acetyl-2,4-di-*O*-benzoyl-α-D-mannopyranosyl)-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-α-D-mannopyranoside (5). A solution of the monohydroxy disaccharide derivative **4** (205 mg, 0.24 mmol) and 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (237 mg, 0.36 mmol) in freshly distilled CH_2Cl_2 (2 mL) was stirred with 4 Å molecular sieves at ~20 °C for 1 h and cooled to 0 °C. Then a solution of AgOTf (0.1 g, ~0.4 mmol) in toluene (2 mL) was added. After 1 h, the reaction mixture was diluted with chloroform. The resulting solution was filtered through a layer of Hyflo super-cel, washed with water and a $Na_2S_2O_3$ solution, and concentrated. Column chromatography afforded fully protected trisaccharide **5** in a yield of 269 mg (78%), $[\alpha]_D$ −43.6 (*c* 0.5). Found (%): C, 66.52; H, 5.01. $C_{79}H_{70}O_{26}$. Calculated (%): C, 66.10; H, 4.92. The NMR spectroscopic data are given in Tables 1 and 2.

Under analogous condensation conditions, **methyl 2,4-di-*O*-benzoyl-6-*O*-(3,6-di-*O*-acetyl-2,4-di-*O*-benzoyl-α-D-mannopyranosyl)-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-[1-^{13}C]mannopyranosyl)-α-D-mannopyranoside (5-^{13}C)** was prepared from disaccharide **4** (180 mg, 0.21 mmol) and 2,3,4,6-tetra-*O*-benzoyl-α-D-[1-^{13}C]mannopyranosyl bromide (210 mg, 0.318 mmol) in a yield of 208 mg (69%).

Methyl 2,4-di-*O*-benzoyl-6-*O*-(2,4-di-*O*-benzoyl-α-D-mannopyranosyl)-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-α-D-mannopyranoside (6). A ~0.6 *M* hydrogen chloride solution (8 mL) in MeOH, which was prepared by the addition of acetyl chloride (0.4 mL) to methanol (10 mL) at 0 °C, was added to a solution of diacetate **5** (110 mg, 0.077 mmol) in CH_2Cl_2 (2 mL). The solution was kept at ~20 °C for ~14 h. After evaporation of the solvent, diol **6** was obtained in a yield of 100 mg (96%), $[\alpha]_D$ −46.6 (*c* 0.7). Found (%): C, 65.96; H, 5.13. $C_{75}H_{66}O_{24}$. Calculated (%): C, 66.66; H, 4.92. The NMR spectroscopic data are given in Tables 1 and 2.

Under analogous conditions, **methyl 2,4-di-*O*-benzoyl-6-*O*-(2,4-di-*O*-benzoyl-α-D-mannopyranosyl)-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-[1-^{13}C]mannopyranosyl)-α-D-mannopyranoside (6-^{13}C)** was prepared from trisaccharide diacetate **5**-^{13}C (188 mg, 0.131 mmol) in a yield of 170 mg (96%).

Methyl 6-*O*-[3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl]-3-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (1). Calcined 4 Å molecular sieves were added to a solution of trisaccharide diol **6** (150 mg, 0.11 mmol) and 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (263 mg, 0.4 mmol) in CH_2Cl_2 (3 mL). The reaction mixture was stirred at 0 °C for 1 h and a solution of AgOTf (257 mg, 0.44 mmol) in toluene (2 mL) was added. Then the reaction mixture was stirred at ~20 °C for 3 h, diluted with chloroform, filtered through a layer of Hyflo super-cel, washed with a $Na_2S_2O_3$ solution, and concentrated. Column chromatography afforded **methyl 2,4-di-*O*-benzoyl-6-*O*-[2,4-di-*O*-benzoyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-α-D-mannopyranoside (7)** in a yield of 202 mg (71.5%), $[\alpha]_D$ −43.5 (*c* 0.5). The NMR spectroscopic data are given in Tables 1 and 2.

The fully protected pentasaccharide (100 mg) was dissolved in dry pyridine (2 mL). Then anhydrous MeOH (1 mL) and 0.5 *M* MeONa (2 mL) were added. The reaction mixture was kept at ~20 °C for 2 days and neutralized with Dowex-50 (H^+) cation-exchange resin, which was prewashed with methanol. The resin was filtered off, the filtrate was concentrated, the residue was dissolved in water, the solution was washed with chloroform, and the aqueous layer was concentrated. The target mannopentaoside was isolated by gel-permeation chromatography (Fractogel TSK HW-40 (S), 1.1×80 cm, 0.1 *M* AcOH as the eluent, a Knauer (Germany) differential refractometer as the detector), the yield was 30 mg (91%), $[\alpha]_D$ +99.8 (*c* 2.0, water) (*cf.* lit data: $[\alpha]_D$ +108.1,[12] +87.5,[14] +100 [20]).

Condensation of trisaccharide glycosyl acceptor **6**-^{13}C (123 mg, 0.091 mmol) with 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (180 mg, 0.273 mmol) in the presence of AgOTf (77 mg, 0.3 mmol) and 4 Å molecular sieves in a mixture of CH_2Cl_2 (2 mL) and toluene (1 mL) was performed analogously to the above-described synthesis of protected mannopentaoside **7** to give **methyl 2,4-di-*O*-benzoyl-6-*O*-[2,4-di-*O*-benzoyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-[1-^{13}C]mannopyranosyl)-α-D-mannopyranoside (7-^{13}C)** in a yield of 160 mg (68%).

Deacylation of this benzoate (50 mg) under the above-described conditions afforded the target **methyl 6-*O*-[3,6-di-**

O-(α-D-mannopyranosyl)-α-D-mannopyranosyl]-3-O-(α-D-[1-^{13}C]mannopyranosyl)-α-D-mannopyranoside (1-^{13}C) in a yield of 7 mg (43%). The ^1H NMR spectrum is shown in Fig. 1.

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