An Efficient Approach towards the Convergent Synthesis of "Fully-Carbohydrate" Mannodendrimers

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Abstract: Glycosylation of sugar trityl ethers with sugar 1,2-*O*-(1-cyano)ethylidene derivatives (the trityl-cyanoethylidene condensation) has been applied to the synthesis of highly branched (dendritic) mannooligosaccharides incorporating a Manα1→3(Manα1→6)Man structural motif. The convergent synthetic strategy used to assemble these oligosaccharides was based on the use of glycosyl acceptors and/or a glycosyl donor already bearing this structural motif. The former were represented by mono- and ditrityl ethers of ManαOMe, Manα1→3ManαOMe, and Manα1→3(Manα1→6)ManαX, where X = OMe or SEt. The pivotal glycosyl donor was the peracetylated 1,2-*O*-(1-cyano)ethylidene-3,6-di-*O*-(α-D-mannopyranosyl)-β-D-mannopyranose (**1**), prepared by orthogonal Helferich glycosylation of the known 1,2-*O*-(1-cyano)ethylidene-β-D-mannopyranose with tetra-*O*-acetyl-α-D-mannopyranosyl bromide followed by O-acetylation. Glycosylation of acetates of methyl 6-*O*-trityl-α-D-mannopyranoside and methyl 3,6-di-*O*-trityl-α-D-mannopyranoside with one equivalent of the donor **1** gave rise to the isomeric tetrasaccharide derivatives, Manα1→3(Manα1→6)Manα1→6ManαOMe and Manα1→3(Manα1→6)Manα1→3ManαOMe, respectively. The latter derivative was further mannosylated at the remaining 6-*O*-trityl acceptor site to give the protected pentasaccharide Manα1→3(Manα1→6)Manα1→3(Manα1→6)ManαOMe. The isomeric pentasaccharide, Manα1→3(Manα1→6)Manα1→6(Manα1→3)ManαOMe, was prepared by reaction of **1** with the 6-*O*-trityl derivative of (Manα1→3)ManαOMe. In a similar fashion, 6′- and 6″-*O*-trityl derivatives of the branched trisaccharide Manα1→3(Manα1→6)ManαOMe served as precursors for two isomeric mannohexaosides. The 3,6-di-*O*-trityl ether of ManαOMe and the 6′,6″-di-*O*-trityl ether of Manα1→3(Manα1→6)ManαX (X = OMe or SEt) were efficiently bis-glycosylated with the donor **1** to give the corresponding protected mannoheptaoside and mannononaoside. The yields of these glycosylations with the donor **1** ranged from 50 to 66%. Final deprotection of all the oligosaccharides was straightforward and afforded the target products in high yields. Both the acylated and deprotected products were characterized, and the intersaccharide connectivities were elucidated by extensive one- and two-dimensional NMR spectroscopy. The described blockwise convergent approach allows assembly of a variety of 3,6-branched mannooligosaccharides.

Keywords: carbohydrates · glycosylation · NMR spectroscopy · oligosaccharides · synthetic methods

Introduction

Carbohydrates, as the most prominent cell surface-exposed structures, play the role of recognition molecules. The message transferred through the sugar code is mainly deciphered[1] in interactions of carbohydrates with proteins, for example, lectins, enzymes, and antibodies. Numerous investigations into relevant ligand–receptor binding events have revealed that the weak binding affinities characteristic of low molecular weight carbohydrates are circumvented in nature through the involvement of multivalent structures. Polyvalent interactions are extremely abundant in biological systems[2] and they often play a crucial role in the binding of carbohydrate ligands to protein receptors.[3] To understand the mechanisms of biological processes mediated by multivalent interactions and to exploit the effects of multivalency,

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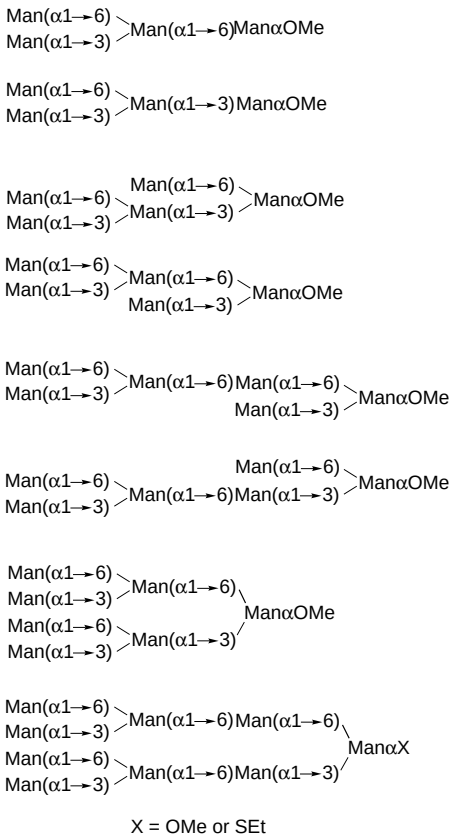
chemists have prepared[4] a variety of ligands with multiple arrays of carbohydrate residues. Artificial multivalent structures can involve, inter alia, glycoside clusters of various architectures,[5] resin-immobilized oligosaccharides,[6] self-assembled carbohydrate-derivatized monolayers,[7] and diverse neoglycopolymers.[8] For some of them, that is to say, for polyvalent mannosides, thermodynamic aspects of binding to concanavalin A have been evaluated.[9]

The family of synthetic multivalent carbohydrate structures has recently been complemented by highly branched (dendritic) oligomers, the so-called glycodendrimers.[10] In addition to *carbohydrate-coated dendrimers* bearing mono- or oligosaccharide terminal groups,[11] and *carbohydrate-centered dendrimers*,[12] yet another group is emerging upon the scene, namely "*fully-carbohydrate*" *glycodendrimers* composed of carbohydrates as building units and constituting the "wedges" of the cascade molecules.[13] Further development of synthetic methodologies for highly branched oligosaccharides is of substantial interest to chemists and glycobiologists.

One of the most rational approaches to higher oligosaccharides composed of *repeating* elements is blockwise synthesis based on the use of the same oligosaccharide glycosyl donor. Here, we describe a blockwise assembly of a number of mannooligosaccharides (Scheme 1) incorporating one, two, or three D-mannopyranose residues glycosylated at positions 3 and 6. The trisaccharide Manα1→3(Manα1→6)Man is a typical branching fragment of mannan chains in N-glycoproteins and the synthesis of some oligosaccharides incorporating this fragment has been reported.[14] Our approach to the construction of branched oligosaccharides is based on a common strategy employing Manα1→3(Manα1→6)Man as a key building block and trityl-cyanoethylidene condensation[15] as the method of glycosylation. The advantages of this glycosylation technique have been demonstrated in the syntheses of several complex, regular polysaccharides of bacterial origin[16] and of regular cyclic oligosaccharides that may be regarded as fully synthetic cyclodextrin analogues.[17]



Scheme 1. Target branched-chain oligomannosides.

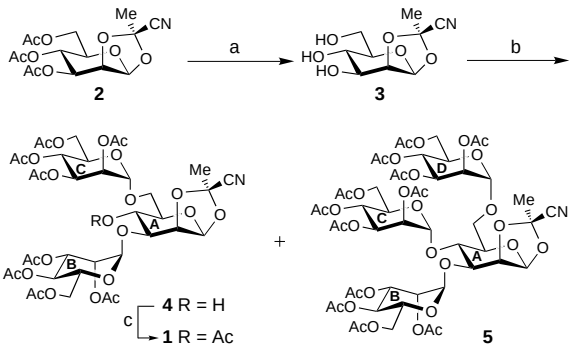
Аннотация: Гликозилирование тритиловых эфиров сахаров 1,2-*O*-(1-циано)этилиденовыми производными углеводов (тритил–цианоэтилиденовая конденсация) было использовано для синтеза высокоразветвленных (дендритных) маннолигосахаридов, включающих структурный элемент Manα1→3(Manα1→6)Man. Конвергентная стратегия синтеза, примененная для сборки этих олигосахаридов, основана на использовании гликозил-акцепторов и/или гликозил-донора, уже содержащих этот элемент. Акцепторами послужили моно- и дитритиловые эфиры ManαOMe, Manα1→3ManαOMe и Manα1→3(Manα1→6)ManαX (X = OMe или SEt). Ключевой гликозил-донор, сполна ацетилированная 1,2-*O*-(1-циано)этилиден-3,6-ди-*O*-(α-D-маннопиранозил)-β-D-маннопираноза (**1**), получена ортогональным гликозилированием по Гельферику известной 1,2-*O*-(1-циано)этилиден-β-D-маннопиранозы тетра-*O*-ацетил-α-D-маннопиранозилбромидом и *O*-ацетилированием. Гликозилирование ацетатов метил-6-*O*-тритил-α-D-маннопиранозида и метил-3,6-ди-*O*-тритил-α-D-маннопиранозида донором **1** (1 экв.) привело к производным изомерных тетрасахаридов, Manα1→3(Manα1→6)Manα1→6ManαOMe и Manα1→3(Manα1→6)Manα1→3ManαOMe, соответственно. Последнее маннозилировали по сохранившейся 6-*O*-тритильной акцепторной группе и получили защищенный пентасахарид Manα1→3(Manα1→6)Manα1→3(Manα1→6)ManαOMe. Изомерный пентасахарид, Manα1→3(Manα1→6)Manα1→6(Manα1→3)ManαOMe, получен реакцией **1** с 6-*O*-тритильным производным (Manα1→3)ManαOMe. Аналогично, 6'- и 6"-*O*-тритильные производные разветвленного трисахарида Manα1→3(Manα1→6)ManαOMe послужили предшественниками двух изомерных гексасахаридов. Бис-гликозилирование 3,6-ди-*O*-тритилового эфира ManαOMe и 6',6"-ди-*O*-тритилового эфира Manα1→3(Manα1→6)ManαX (X = OMe или SEt) донором **1** привело к соответствующим защищенным манногепта- и -нонаозидам. Выходы при гликозилировании донором **1** составляли от 50 до 66%. Удаление защитных групп привело к целевым соединениям с высокими выходами. Анализ структуры ацилированных и свободных олигосахаридов проводили с помощью одно- и двумерной спектроскопии ЯМР. Описанный блочный конвергентный подход позволяет синтезировать разнообразные 3,6-разветвленные маннолигосахариды.

Ключевые слова: углеводы, гликозилирование, олигосахариды, синтетические методы.

Results and Discussion

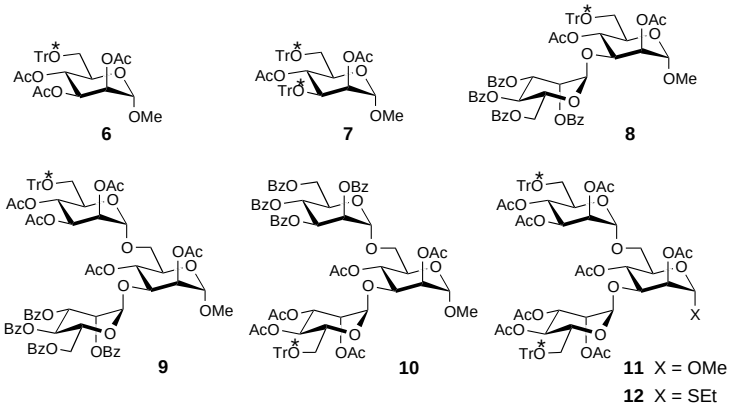
Synthesis of the glycosyl donor 1: A convergent approach to the synthesis of mannooligosaccharides incorporating 3,6-branched fragments requires a highly efficient and readily accessible donor based on a Manα1→3(Manα1→6)Man trisaccharide. Several synthetic schemes have been elaborated[14b, 18–20] for the construction of protected derivatives of branched mannooligosaccharides, including those that can act as glycosyl donors.[21, 22] Compound **1** (Scheme 2) fulfils these requirements since 1,2-*O*-cyanoethylidene derivatives of saccharides are known[15] to be excellent 1,2-*trans*-glycosylating agents. In our synthesis of **1**, we converted the known[23] 1,2-*O*-[1-(*exo*-cyano)ethylidene]-β-D-mannopyranose triacetate **2** into the triol **3** and glycosylated it selectively at positions 3 and 6.

The transformation of **2** into the triol **3** had previously been accomplished by Et_3N-[17] or NaOMe-catalyzed[24] methanolysis. Here, we effected the deacetylation with NaOMe in a $MeOH/C_5H_5N$ mixture,[25] and the product **3** was then subjected to glycosylation. Glycosylation of hydroxyl-con-



Scheme 2. Synthesis of the trisaccharide glycosyl donor **1**. Reagents and conditions: a) NaOMe/MeOH/C_5H_5N, room temperature, 5 min; b) 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide, $Hg(CN)_2$, $HgBr_2$, MeCN, room temperature, 16 h (with 3.5 equiv of the glycosyl bromide the product is **4**, 62%; with 4 equiv of the glycosyl bromide, the products after acetylation are **1**, 46%, and **5**, 16%); c) Ac_2O/C_5H_5N, DMAP, 80%.

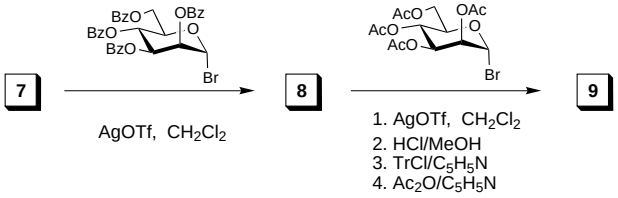
taining cyanoethylidene derivatives, which may be considered as an example of orthogonal glycosylation,[26] has substantially extended the potential of the trityl-cyanoethylidene condensation. This reaction is usually performed with acylglycosyl bromides under the conditions of the Helferich reaction[27] or in the presence of silver trifluoromethanesulfonate (triflate) in combination with 2,4,6-collidine.[17] To ensure selective glycosylation of the triol **3**, we employed a milder promoter, namely, a mixture of $Hg(CN)_2$ and $HgBr_2$. The reaction of triol **3** with 3.5 equivalents of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide resulted in the formation of trisaccharide **4** (62% yield after chromatography). Acetylation of this cyanoethylidene derivative afforded the crystalline fully protected compound **1**. When a somewhat larger amount of the glycosyl bromide (4 equivalents) was used, a tetrasaccharide **5** was obtained together with the trisaccharide **4**. Since both these saccharides exhibit nearly identical chromatographic mobilities on silica gel, their separation was effected by chromatography after acetylation. The yield of the target compound **1** was 46%, while that of compound **5** was 16%. A similar approach to the construction of oligosaccharides incorporating 3,6-bis-glycosylated hexopyranose residues, but requiring a larger number of steps, has been reported previously.[28]



Scheme 3. Building blocks containing trityl ethers that can serve as glycosyl acceptors in reactions with the trisaccharide donor **1**. The potential sites of glycosylation are marked with asterisks.

The glycosyl acceptors 6–12: To construct the required variety of branched oligosaccharides (Scheme 1), it was necessary to synthesize a range of saccharide derivatives that could serve as glycosyl acceptors for the trisaccharide donor **1** in the trityl-cyanoethylidene condensation. To this end, we used the known trityl ethers **6**[29] and **7**[30] of methyl α-D-mannopyranoside, as well as compounds **8**–**12**[31] (Scheme 3).

In summary, the trityl ether **8** (Scheme 4) was prepared by selective glycosylation of the primary–secondary trityl ether **7** by 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide at



Scheme 4. Synthesis of the trityl ethers **8** and **9**.

the secondary position.[32] This disaccharide **8** was further glycosylated by 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide to give a trisaccharide with differently protected mannopyranosyl residues. Its de-O-acetylation by mild acid-catalyzed methanolysis, which did not affect the O-benzoyl groups,[33] followed by 6-O-tritylation ($TrCl/C_5H_5N$) and O-acetylation, furnished the trisaccharide trityl ether **9**. The trisaccharide trityl ether **10**, isomeric with compound **9**, was synthesized from **7** according to an analogous route, except that the sequence of attachment of the O-acetylated and O-benzoylated mannose units to the acceptor **7** was reversed. The tetra-*O*-acetyl-α-D-mannopyranosyl residue was first introduced[32a] at position 3 of the acceptor, and then the resulting 6-*O*-trityl ether was subjected to AgOTf-promoted mannosylation with 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide. The peracylated trisaccharide thus formed was modified as outlined above (selective de-O-acetylation, O-tritylation, and O-acetylation) to yield the target trityl ether **10**. Syntheses of the bis-trityl ethers **11** and **12** involved bis-glycosylation of the ditrityl ether **7** (or its 1-SEt analogue), complete deacylation of the peracylated trisaccharide derivatives, tritylation of the primary OH groups, and per-O-acetylation.

Syntheses of the oligosaccharides: Previous glycosylations by cyanoethylidene derivatives of disaccharides[16c,d] and linear tri-[16b] and tetrasaccharides[34] have shown that their efficiencies do not differ from those of monosaccharide derivatives. One might expect the cyanoethylidene derivative of a branched trisaccharide, such as compound **1**, to also be an efficient glycosyl donor. To verify this hypothesis, we carried out a reaction of **1** with methyl 2,3,4-tri-*O*-acetyl-6-*O*-trityl-α-D-mannopyranoside **6** as a glycosyl acceptor (Scheme 5). This reaction was conducted under the standard conditions of trityl-cyanoethylidene condensation,[15] and afforded the fully protected tetrasaccharide derivative **13** in 56% yield. Thus, the 1,2-*O*-cyanoethylidene derivative of a branched trisac-

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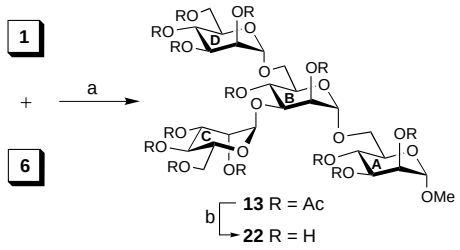
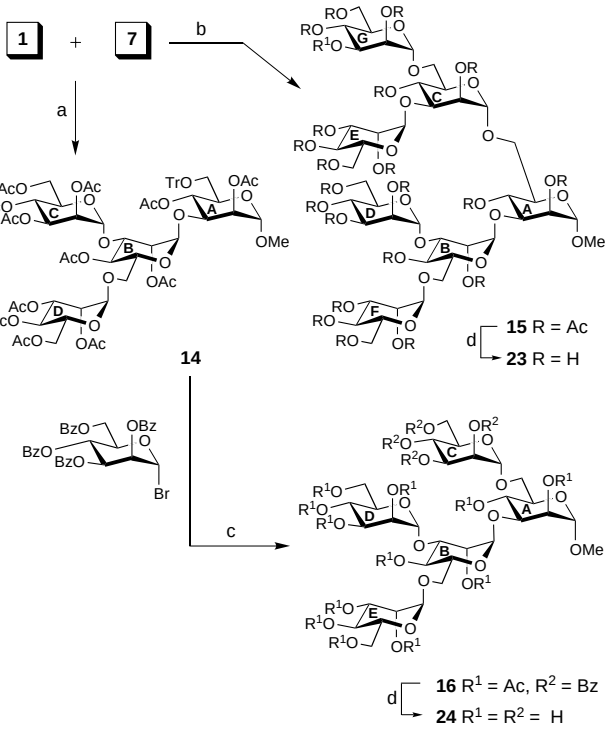


Scheme 5. Synthesis of the tetrasaccharide **13** (and **22**). Reagents and conditions: a) $TrClO_4$ (10 mol%), CH_2Cl_2, room temperature, 16 h, 56%; b) NaOMe/MeOH/C_5H_5N.

charide, such as compound **1**, can indeed be used as a building block for the construction of complex oligomannosides.

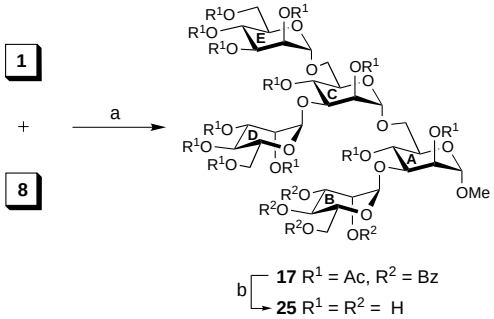
The next step in studying the reactivity of compound **1** as a glycosyl donor was an investigation of its reactions with the ditrityl ether **7**, which can be selectively monoglycosylated at position 3[31, 32] or bis-glycosylated at positions 3 and 6[31] with monosaccharide glycosyl donors. Here (Scheme 6), glycosylation of compound **7** with one equivalent of the glycosyl



Scheme 6. Synthesis of the tetrasaccharide **14**, heptasaccharide **15** (and **23**), and pentasaccharide **16** (and **24**). Reagents and conditions: a) **1** (1 mol equiv), $TrClO_4$ (10 mol%), CH_2Cl_2, room temperature, 16 h, 55%; b) **1** (2 mol equivalents), $TrClO_4$ (10 mol%), CH_2Cl_2, room temperature, 16 h, 60%; c) 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (4 equiv), AgOTf/2,4,6-collidine, CH_2Cl_2, room temperature, 10 min (47%); d) NaOMe/MeOH/C_5H_5N.

donor **1** gave the tetrasaccharide derivative **14** in 55% yield. Owing to the lower reactivity of primary trityl ethers as compared with their secondary counterparts, the 6-*O*-trityl group remained unchanged in the molecule. The use of a twofold molar excess of the donor **1** for the glycosylation of the acceptor **7** led to the formation of the heptasaccharide derivative **15**, which was isolated in about 60% yield. The tetrasaccharide **14** was used as a glycosyl acceptor for the synthesis of a mannopentaoside under the conditions of the Bredereck reaction. Condensation of **14** with 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide in the presence of silver triflate and 2,4,6-collidine (Scheme 6) gave the protected pentaoside **16** in 47% yield.

The demonstrated effectiveness of the use of trisaccharide **1** as a glycosyl donor, in combination with 6-*O*- and 3,6-di-*O*-trityl ethers of mannopyranose as glycosyl acceptors, served as a basis for the blockwise synthesis of other branched oligomannosides. The aforementioned glycosyl acceptors **8–12** were used for this purpose. The positions of the *O*-trityl groups in compounds **8–12** dictate the sites for the introduction of the Manα1→3(Manα1→6)Man fragment. Thus, condensation of the glycosyl donor **1** with the disaccharide acceptor **8** under the standard conditions for the trityl-cyanoethylidene condensation gave (Scheme 7) the peracylated pentaoside **17** in 66% yield following chromatography.

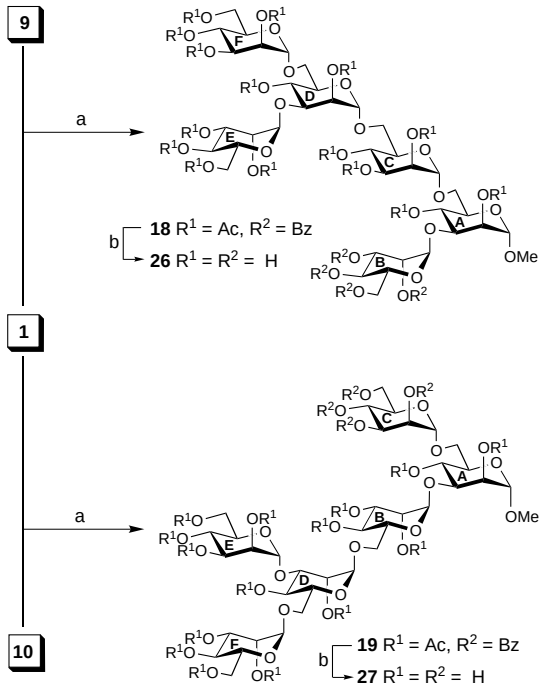


Scheme 7. Synthesis of the pentaoside **17** (and **25**). Reagents and conditions: a) $TrClO_4$ (10 mol%), CH_2Cl_2, room temperature, 16 h, 66%; b) NaOMe/MeOH/C_5H_5N.

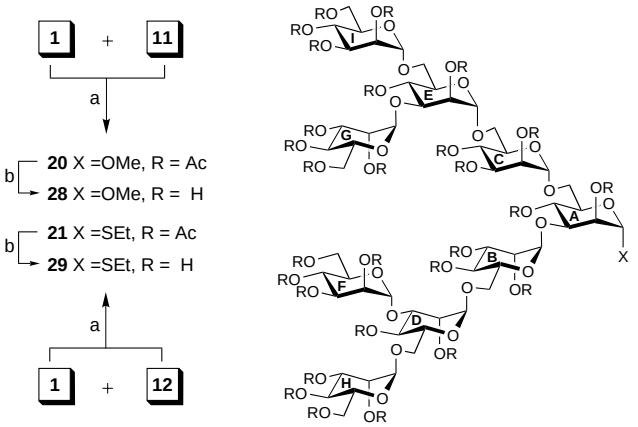
Under similar conditions, glycosylation of the trityl ethers **9** and **10** with equivalent amounts of the cyanoethylidene derivative **1** (Scheme 8) yielded the fully protected hexaosides **18** (58%) and **19** (60%). The bis-trityl ethers **11** and **12** underwent bis-glycosylation with the donor **1** to give (Scheme 9) nonaosides **20** (50%) and **21** (54%), respectively. The formation of the nonaoside **21** demonstrates the stability of thioglycosides under the conditions of the trityl-cyanoethylidene condensation and represents yet another example of orthogonal glycosylation. The structures of all the oligosaccharides obtained followed unambiguously from the manner of their synthesis and were confirmed by ^1H and ^{13}C NMR spectroscopy and mass spectrometry.

All peracylated oligosaccharides were deprotected (Zemplén) to give the unprotected tetraoside **22** (Scheme 5), the "symmetrical" heptaoside **23**, the known[35] isomeric pentaosides **24** (Scheme 6) and **25** (Scheme 7), the isomeric hexaosides **26** and **27** (Scheme 8), and the nonaosides **28** and **29** (Scheme 9) in virtually quantitative yields. The ^1H and ^{13}C NMR spectra of these oligosaccharides corroborate their structures.

NMR spectroscopy: The branched mannooligosaccharides, both protected and unprotected, were characterized by means



Scheme 8. Synthesis of the isomeric hexaosides **18** and **19** (and **26** and **27**). Reagents and conditions: a) $TrClO_4$ (10 mol%), CH_2Cl_2, room temperature, 16 h, 58% (**18**) and 60% (**19**); b) NaOMe/MeOH.



Scheme 9. Synthesis of the nonaosides **20** and **21** (and **28** and **29**). Reagents and conditions: a) $TrClO_4$ (10 mol%), CH_2Cl_2, room temperature, 16 h, 50% (**20**) and 54% (**21**); b) NaOMe/MeOH/C_5H_5N.

of 1D- and 2D NMR spectroscopy. For most of the new compounds, all peaks in the ^1H and ^{13}C NMR spectra could be assigned to such an extent that it was possible 1) to delineate the spin system for each *individual* α-D-mannopyranose residue, and 2) to establish intersaccharide *connectivities*. The spin-spin coupling constants of vicinal protons available from 1D ^1H NMR spectra had values indicative of α-D-mannopyranose residues ($J_{1,2} \approx 1.5-2.0$ Hz; $J_{2,3} \approx 3.0-3.5$ Hz), but their utility in making signal assignments was limited. Correlation spectroscopy (COSY),[36] relayed coherence transfer spectroscopy (COSYRCT),[37] and total correlation spectroscopy (TOCSY)[38] were used for identifying groups of protons belonging to separate mannopyranosidic residues.

Assignments of the ^1H NMR spectra of the protected pentasaccharides **16** and **17** and hexasaccharides **18** and **19** were facilitated by the fact that the signals of the protons of the monosaccharide residues bearing *O*-benzoyl groups are shifted downfield compared to those of the other protons. In this respect, the ^1H NMR spectrum of compound **19** (Figure 1) is noteworthy as all the signals of the protons of residue **C** are distinguishable, even in the 1D spectrum. The chemical shifts of the protons of the other monosaccharide residues in this oligosaccharide proved to be sufficiently different to permit their assignments using a combination of 2D ^1H NMR spectra.

The relationships between sets of signals belonging to glycosidically linked residues (in this work designated as **A**, **B**, **C**, and so on, as shown in the schemes) were established using either the 1D NOE technique in a difference mode with preirradiation of the anomeric protons and/or rotating-frame Overhauser enhancement spectroscopy (ROESY).[39] For instance, the glycoside residues (residues **A**) were unequivocally identified from the H-1**A**/OMe correlations in the ROESY spectra. Analysis of the ROESY spectra of the deprotected oligosaccharides revealed, as expected, the presence of H-1′/H-6 and H-1′/H-3 cross-peaks for the Man1 → 6Man and Man1 → 3Man fragments, respectively. For the Man1 → 3Man fragment, H-1′/H-2 and H-1′/H-4 cross-peaks were also observed, probably as a result of spin diffusion and/or spatial transfer.[40] Heteronuclear multiple quantum coherence (HMQC)[41] spectroscopy proved to be useful for the unambiguous identification of anomeric *protons* in ^1H NMR spectra from the characteristic, low-field resonances of the anomeric *carbon* atoms. For instance, the high-field chemical shift ($\delta_C = 86.3$ ppm) of the anomeric carbon atom of residue **A** bearing the ethylthio group in the nonaoside **29** allowed us to identify ($\delta_H = 5.44$ ppm) the anomeric proton in this residue using the HMQC procedure (Figure 2). In this way, an independent and comprehensive proof of the relative positions of each residue in an oligosaccharide framework was obtained for all the synthesized compounds.

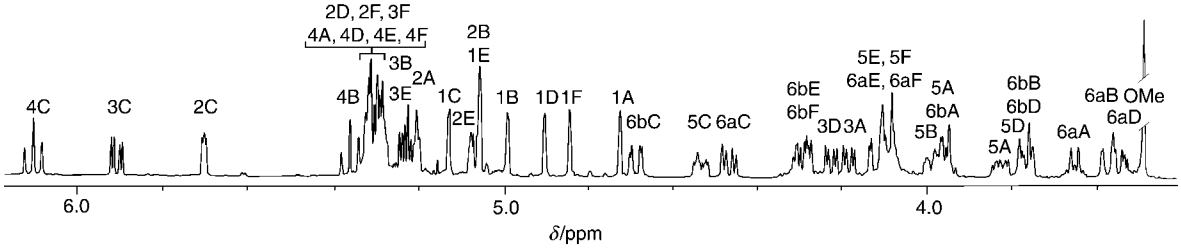


Figure 1. The "carbohydrate region" of the ^1H NMR spectrum (500 MHz, $CDCl_3$, 20 °C) of the hexasaccharide derivative **19** showing the assignments of all the resonances. The designation of the monosaccharide residues (**A**–**F**) in **19** is shown in Scheme 8.

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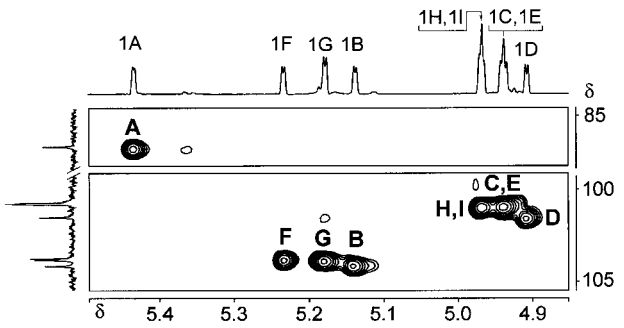

Figure 2. The anomeric region of the 2D-transformed data matrix from an HMQC experiment conducted on the nonasaccharide **29** (100 MHz, D_2O, 25 °C). The assignment A/B/C/D/E/F/G/H/I of the D-mannopyranose residues is illustrated in Scheme 9.

Table 1. Types of α-D-mannopyranose residues that can be identified within the structures of the synthesized mannooligosaccharides.[a]

Residues	Protected		Deprotected	
terminal	$Ac_4Man(1\rightarrow3)$	(11)	$Man(1\rightarrow3)$	(13)
	$Ac_4Man(1\rightarrow6)$	(10)	$Man(1\rightarrow6)$	(13)
	$Bz_4Man(1\rightarrow3)$	(2)		
	$Bz_4Man(1\rightarrow6)$	(2)		
6-monosubstituted	$\rightarrow6)Ac_3Man(1\rightarrow3)$	(3)	$\rightarrow6)Man(1\rightarrow3)$	(3)
	$\rightarrow6)Ac_3Man(1\rightarrow6)$	(3)	$\rightarrow6)Man(1\rightarrow6)$	(3)
3,6-disubstituted	$\rightarrow3,6)Ac_2Man(1\rightarrow3)$	(3)	$\rightarrow3,6)Man(1\rightarrow3)$	(2)
	$\rightarrow3,6)Ac_2Man(1\rightarrow6)$	(9)	$\rightarrow3,6)Man(1\rightarrow6)$	(9)
'reducing'	$\rightarrow3,6)Ac_2Man(1\rightarrow OMe$	(6)	$\rightarrow3,6)Man(1\rightarrow OMe$	(6)

[a] Values in parentheses represent the numbers of similar residues.

The signals of the carbon atoms were assigned by means of HMQC and the attached proton test (APT) technique,[42] taking into account the characteristic downfield shifts, that is, the α-glycosylation effects,[43] for C-3 and/or C-6 bearing a carbohydrate substituent as compared with those of the corresponding non-glycosylated carbon atoms. Thus, the fact that the C-3 atoms of the 3-substituted residues, as well as the C-6 atoms, can be reliably identified in the ^{13}C NMR spectra of deprotected glycosides **22**–**29** allowed us to use the HMQC and APT techniques to assign the H-3 and H-6 signals in the ^1H NMR spectra of these oligosaccharides. The chemical shifts of all other carbon atoms were determined from the assigned signals for the protons.

The chemical shifts of the anomeric H and C atoms of the methyl pentaosides **24** and **25** are consistent with those reported in the literature.[35] The $^1J_{C1,H1}$ coupling constants (GATED spectrum) determined for compound **22** are in the range 171–173 Hz, an observation that establishes α-configurations for the glycosidic linkages in all of the monosaccharide residues.[44]

The assignment of all ^{13}C signals of the heptasaccharide **15** was not possible because of the similarities of the chemical shifts of the protons for similar structural units (for example, **D** and **E**, **F** and **G**). However, the structurally significant resonances, that is to say, those of the anomeric carbons, the C-6 atoms, and the low-field resonances of the C-3 atoms of the units **A**, **B**, and **C**, could be assigned. Our interpretations of the NMR spectroscopic data for the protected mannooligosaccharides based on the described methodology are summarized in the tables in the Experimental Section. The interpreted NMR spectra allowed us to summarize the characteristic spectroscopic features of the branched oligosaccharides studied. The structural elements incorporated into the newly synthesized oligosaccharides can be grouped according to the type of substitution on an α-D-mannopyranose residue (Table 1). As expected, the NMR spectra of similar mannopyranose residues are nearly identical, whereas different types of residues give rise to noticeable differences. Thus, a comparison of the NMR spectroscopic characteristics of the different structural elements revealed features typical of the protected and deprotected 3,6-branched mannooligosaccharides. To this end, the chemical shifts of the analogous atoms present in similar mannopyranose residues were averaged and the resulting mean chemical shifts are schematically depicted in Figures 3 and 4.

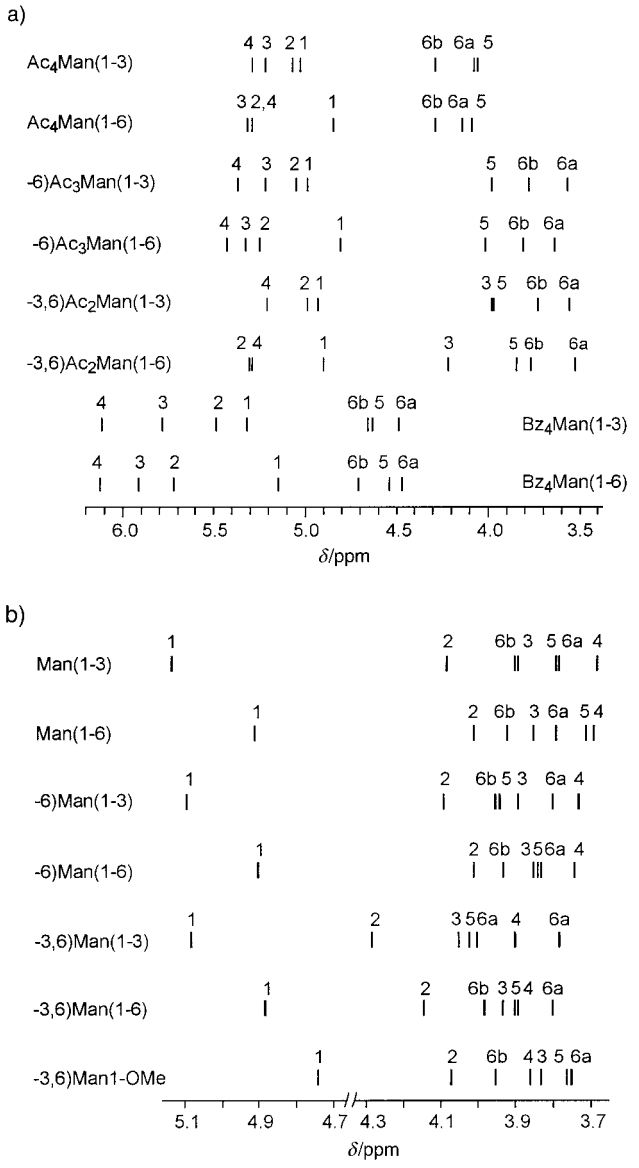

Figure 3. A schematic representation of ^1H NMR spectroscopic data for some typical α-D-mannopyranose residues incorporated into a) protected and b) deprotected 3,6-branched mannooligosaccharides. Chemical shifts are depicted as vertical lines and their values are calculated as the means of δ values for the analogous signals in similar residues selected from Tables 2 and 4.

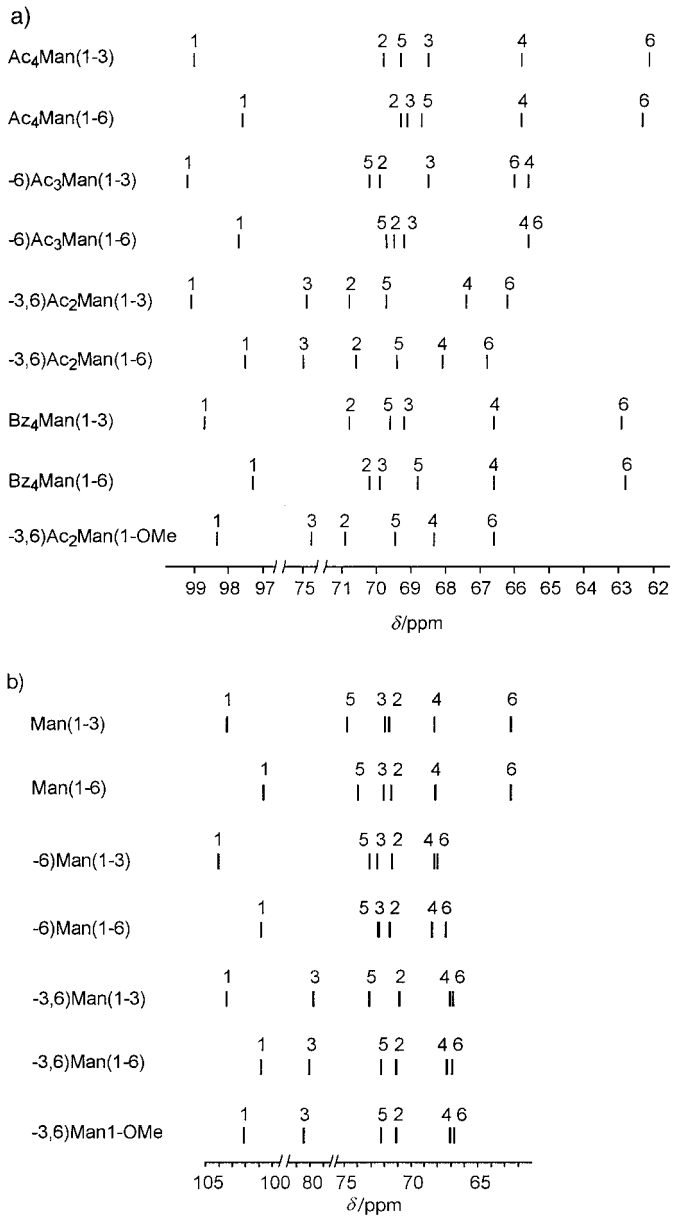

Figure 4. A schematic representation of ^{13}C NMR spectroscopic data for some typical α-D-mannopyranose residues incorporated into a) protected and b) deprotected 3,6-branched mannooligosaccharides. Chemical shifts are depicted as vertical lines and their values are calculated as the means of δ values for the analogous signals in similar residues selected from Tables 3 and 5.

Figure 3a shows the mean ^{1}H NMR parameters for the protected oligosaccharides. The chemical shifts for H-3 and/or H-6a and H-6b are shifted upfield upon *O*-3 and *O*-6 glycosylation. The effect of the linkage type manifests itself most distinctly and systematically in the chemical shift differences for H-2 ($\Delta\delta = +0.20$ to $+0.23$ ppm) and H-1 ($\Delta\delta = -0.17$ to -0.18 ppm, except at the branching points) of the glycosylating residues on going from the $(1\rightarrow3)$- to the $(1\rightarrow6)$-linkage. The chemical shifts of H-1 of the glycoside residues have an average value of $\delta = 4.69$ ppm, while the chemical shifts of the other protons of these residues depend on the type of acyl protecting groups on the attached residues, and have therefore not been averaged.

The ^{1}H NMR spectra of the deprotected oligosaccharides (Figure 3b) also display several linkage type dependent chemical shifts. Upfield shifts on going from the $(1\rightarrow3)$- to the $(1\rightarrow6)$-linkage are observed for the anomeric protons ($\Delta\delta = -0.19$ to -0.22 ppm) and H-5 atoms ($\Delta\delta = -0.08$ to -0.10 ppm). The resonances of the H-2 atoms are somewhat more downfield shifted in the $(1\rightarrow3)$-linked residues as compared with the $(1\rightarrow6)$-linked ones ($\Delta\delta = -0.07$ to -0.14 ppm).

The ^{13}C NMR spectra seem to be more sensitive to the type of residue within the 3,6-branched mannooligosaccharide structures than the ^{1}H NMR spectra. The α-glycosylation effects are well defined (Figure 4a), corresponding to $+5.8$ ppm (C-3) and $+3.3$ ppm (C-6) for the $(1\rightarrow6)$-linked units and $+6.4$ ppm (C-3) and $+3.9$ ppm (C-6) for the $(1\rightarrow3)$-linked ones. These characteristic shift differences have successfully been employed in the interpretation of the ^{13}C NMR spectra of the synthesized protected oligosaccharides. The resonances of C-1, C-2, and C-5 of the $(1\rightarrow6)$-linked units are somewhat upfield shifted compared with the $(1\rightarrow3)$-linked ones, these shifts being largest for anomeric carbon atoms and ranging from -1.4 to -1.6 ppm.

The ^{13}C NMR spectroscopic pattern of the monosaccharide units of deprotected oligosaccharides (Figure 4b) is very similar to that of the protected oligosaccharides. Thus, the resonances of the anomeric carbon atoms involved in $1\rightarrow3$ linkages are shifted downfield by 2.6–3.2 ppm compared with those involved in $1\rightarrow6$ linkages. The α-glycosylation effects are $+7.1$ to $+7.6$ ppm for C-3 and $+5.5$ to $+4.9$ ppm for C-6. No remarkable β-glycosylation effect is observed for C-4 in branching monosaccharide units. The resonances of C-4 in these units are shifted upfield by 1.1–1.2 ppm compared to those observed in non-substituted (terminal) or 6-substituted monosaccharide residues.

Thus, NMR spectroscopy can be used with confidence for the characterization of these oligosaccharides containing similar structural elements. It is also possible to determine both the total number of anomeric H and C atoms and the ratio of the low-field $(1\rightarrow3$ bond) to the high-field $(1\rightarrow6$ bond) signals, which gives an indication of the degree of branching in the newly synthesized oligosaccharides.

Conclusion

The results reported herein concerning the synthesis of highly branched mannooligosaccharides (5-mers, 6-mers, a 7-mer, and a 9-mer) demonstrate the efficiency of the blockwise synthetic approach and hence one may anticipate its successful application in the synthesis of more complex dendritic carbohydrate structures. Triphenylmethylium perchlorate catalyzed condensation of tritylated oligosaccharides (glycosyl acceptors) with a branched cyanoethylidene derivative (glycosyl donor) has enabled the stereospecific and regiospecific introduction of Manα1→3(Manα1α→6)Man fragment(s) at the site(s) of tritylation on the acceptors. In the case of dityrityl ethers, two trisaccharide fragments can be introduced simultaneously or, if the acceptor bears both primary and secondary *O*-trityl groups, regioselectively at the

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secondary position. The branched oligosaccharides obtained may be regarded as first and second generation glycodendrons.

Experimental Section

General information and techniques: Triphenylmethylium perchlorate ($TrClO_4$) was prepared according to the published procedure[45] and reprecipitated from a solution in nitromethane with dry diethyl ether;[46] silver trifluoromethanesulfonate (AgOTf) was prepared as described elsewhere.[47] Pyridine was distilled from KOH. Dichloromethane and acetonitrile were distilled from P_2O_5 and CaH_2, nitromethane from CaH_2, and all were stored over 3 Å molecular sieves. The solvents used in the trityl-cyanoethylidene condensation (benzene and dichloromethane) were degassed and distilled over CaH_2 in a high-vacuum system. Solutions were concentrated at about 40 °C on a rotary evaporator. Glycosylations of the trityl ethers **6–12** with the cyanoethylidene derivative **1** were carried out by applying vacuum techniques.[15] Column chromatography was carried out using Silpearl silica gel (Sklarny Kavalier, Czech Republic). Thin-layer chromatography (TLC) was carried out on Merck DC-Alufolien Kieselgel 60F254. Spots were visualized by spraying with 25% H_2SO_4 and subsequent heating at about 150 °C. Tritylated compounds gave a bright-yellow coloration immediately after spraying or on gentle heating. Melting points were determined on a Kofler hot stage. Optical rotations were measured using a JASCO DIP-360 polarimeter at about 20 °C in chloroform (protected oligosaccharides) or water (deacylated oligosaccharides). Fast atom bombardment mass spectra were obtained with a Kratos MS80RF instrument using a krypton primary atom beam at 8 eV and *trans*-indol-3-ylacrylic acid or 3-nitrobenzyl alcohol matrices. Time-of-flight mass spectrometry with matrix-assisted laser-desorption ionization was carried out on a Vision 2000 instrument. ^1H and ^{13}C NMR spectra were recorded on Bruker WM-250, AM-300, and DRX-500 instruments with samples in $CDCl_3$ (protected oligosaccharides, Me_4Si as the internal standard) and D_2O (deacylated oligosaccharides, acetone as the internal standard, $\delta_H = 2.225$ ppm, $\delta_C = 31.45$ ppm). 2D NMR spectra were obtained using standard Bruker software for Aspect 2000 and 3000 spectrometers (APT, COSY, COSYRCT, ROESY, HMQC). NMR data for compounds **1**, **4**, **5**, **13–29** are listed in Tables 2–5.

1,2-*O*-[1-(*exo*-Cyano)ethylidene]-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-mannopyranose (4): A mixture of 1,2-*O*-[1-(*exo*-cyano)ethylidene]-β-D-mannopyranose[25] (**3**; 1.95 g, 8.44 mmol), $Hg(CN)_2$ (7.60 g, 30.0 mmol), and $HgBr_2$ (1.08 g, 3.00 mmol) was dried in vacuo (oil pump) for 2 h. MeCN (10 mL) was added, and then a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide[24] (12.33 g, 30.0 mmol) in MeCN (15 mL) was added dropwise with stirring over a period of about 1 h. Stirring was continued for about 16 h at ambient temperature. The reaction mixture was then concentrated, the residue was partitioned between $CHCl_3$ and aqueous NaI (about 100 mL of each), and the organic layer was washed with water and concentrated. Column chromatography (PhMe/EtOAc, 1:1) afforded the product **4** (4.7 g, 62%), $[\alpha]_D = +35.4$ ($c = 0.88$); elemental analysis calcd (%) for $C_{37}H_{49}NO_{24}$ (891.8): C 49.83, H 5.53, N 1.57; found: C 49.92, H 5.62, N 1.50.

4-*O*-Acetyl-1,2-*O*-[1-(*exo*-cyano)ethylidene]-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-mannopyranose (1) and 1,2-*O*-[1-(*exo*-cyano)ethylidene]-3,4,6-tri-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-mannopyranose (5): a) The monohydroxy derivative **4** (1.0 g) was acetylated with Ac_2O (2 mL) in C_5H_5N (1 mL) in the presence of a catalytic amount of DMAP at about 20 °C for 16 h. After cooling, several drops of H_2O were added to the mixture, which was then diluted with $CHCl_3$. The resulting solution was successively washed with H_2O, dilute aqueous HCl, and aqueous $NaHCO_3$, and concentrated. Crystallization of the residue from MeOH gave the title compound **1** (0.84 g, 80%); m.p. 154–159 °C; $[\alpha]_D = +27.3$ ($c = 0.9$); elemental analysis calcd (%) for $C_{39}H_{51}NO_{25}$ (933.8): C 50.16, H 5.50, N 1.50; found: C 50.31, H 5.17, N 1.25.

b) Glycosylation of **3** (3.85 g, 16.7 mmol) with 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (27.4 g, 66.8 mmol) in the presence of $Hg(CN)_2$ (16.9 g, 66.8 mmol) and $HgBr_2$ (2.11 g, 5.85 mmol) in MeCN (20 mL) was carried out as described above. Column chromatography gave a fraction

Table 2. Chemical shifts (δ values) of the mannopyranosidic protons in the ^1H NMR spectra ($CDCl_3$, 25 °C) of protected mannooligosaccharides **1**, **4**, **5**, and **13–21**.

Compound[a]		H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
1	A	5.46	4.63	3.96	5.16	3.61	3.52	3.72
	B	4.95	5.08	5.27	5.28	4.17	4.08	4.32
	C	4.74	5.19	5.26	5.26	4.00	4.00	4.27
4	A	5.42	4.64	3.80	3.96	3.46	3.73	3.97
	B	5.09	5.40	5.36	5.36	4.27	4.14	4.36
	C	4.84	5.26	5.28	5.28	4.06	4.12	4.27
5	A	5.49	4.73	3.98	3.91	3.66	3.82	3.90
	B	5.07	5.30	5.30	5.29	4.21	4.12	4.33
	C	5.14	5.20	5.24	5.27	3.97	4.11	4.29
	D	4.89	5.26	5.29	5.32	4.04	4.08	4.27
13	A	4.65	5.20	5.32	5.30	3.90	3.56	3.72
	B	4.86	5.24	4.15	5.22	3.78	3.48	3.72
	C	4.97	5.02	5.16	5.26	4.05	4.05	4.25
	D	4.79	5.22	5.30	5.25	4.05	4.05	4.23
14	A	4.73	5.15	4.10	5.15	3.79	3.12	3.19
	B	4.86	4.93	3.96	5.18	3.94	3.54	3.71
	C	4.94	5.04	5.17	5.22	3.90	4.00	4.28
	D	4.88	5.35	5.36	5.27	4.05	4.10	4.29
15	A	4.64	5.13	4.14	5.20	3.80	3.50	3.74
	B	4.92	4.98	3.95	5.20	3.94	3.53	3.71
	C	4.84	5.23	4.13	5.26	3.84	3.51	3.74
	D	4.94	5.04	5.19	5.25	3.93	3.99	4.30
	E	4.96	5.03	5.17	5.25	4.03	4.03	4.25
	F	4.88	5.33	5.33[b]	5.27[b]	4.03	4.08	4.29
	G	4.82	5.25	5.28	5.26	4.07	4.10	4.26
16	A	4.73	5.21	4.24	5.27	3.96	3.66	3.93
	B	4.99	5.04	4.01	5.23	3.99	3.57	3.75
	C	5.14	5.71	5.90	6.11	4.52	4.46	4.70
	D	4.99	5.08	5.19	5.27	3.96	4.04	4.31
	E	4.90	5.37	5.38	5.29	4.07	4.12	4.31
17	A	4.71	5.41	4.33	5.43	3.85	3.56	3.82
	B	5.30	5.47	5.78	6.10	4.62	4.45	4.63
	C	4.89	5.29	4.23	5.30	3.95	3.53	3.79
	D	5.03	5.08	5.23	5.32	4.12	4.15	4.28
	E	4.85	5.30	5.33	5.32	4.09	4.15	4.29
18	A	4.69	5.43	4.35	5.29[b]	3.88	3.50	3.82
	B	5.32	5.48	5.76	6.10	4.62	4.51	4.67
	C	4.82[c]	5.28	5.36	5.42	4.06	3.65	3.83
	D	4.93	5.32	4.23	5.30[b]	3.86	3.53	3.80
	E	5.05	5.09	5.24	5.31[b]	4.11	4.06	4.30
	F	4.84[c]	5.29	5.33	5.30[b]	4.10	4.13	4.27
19	A	4.73	5.21	4.20	5.30	3.97	3.66	3.97
	B	5.00	5.07	5.24	5.37	4.00	3.58	3.78
	C	5.14	5.71	5.91	6.11	4.55	4.47	4.70
	D	4.91	5.33	4.24	5.32	3.84	3.56	3.78
	E	5.07	5.08	5.23	5.30	4.11	4.11	4.30
	F	4.85	5.30	5.31	5.31	4.10	4.13	4.30
20	A	4.67	5.16	4.12	5.16	3.83	3.49	3.78
	B	4.97	5.02	5.20	5.37	3.97	3.57	3.76
	C	4.81	5.23	5.32	5.42	4.00	3.62	3.80
	D	4.90	5.32	4.22	5.30	3.83	3.54	3.77
	E	4.93	5.31	4.22	5.29	3.85	3.53	3.77
	F	5.06	5.09	5.23	5.30	4.09	4.10	4.29
	G	5.05	5.09	5.23	5.30	4.09	4.10	4.29
	H	4.83	5.29	5.30	5.30	4.09	4.14	4.28
	I	4.84	5.29	5.30	5.30	4.09	4.14	4.28
21	A	5.26	5.26	4.13	5.17	4.27	3.45	3.79
	B	4.96	5.02	5.18	5.34	3.95	3.52	3.76
	C	4.78	5.21	5.27	5.42	3.96	3.62	3.77
	D	4.86	5.33	4.25	5.28	3.82	3.54	3.76
	E	4.93	5.31	4.21	5.27	3.82	3.51	3.76
	F	5.06	5.07	5.21	5.28	4.09	4.10	4.27
	G	5.04	5.07	5.22	5.29	4.09	4.10	4.29
	H	4.84	5.28	5.31	5.28	4.08	4.12	4.28
	I	4.83	5.27	5.31	5.28	4.08	4.13	4.27

[a] The rows correspond to individual α-D-mannopyranose residues designated as **A**, **B**, **C**, etc. (see schemes). [b,c] Assignment within the group of signals may be interchanged.

Table 3. Chemical shifts (δ values) of the mannopyranosidic carbons in the ^{13}C NMR spectra ($CDCl_3$, 25°C) of protected mannooligosaccharides **1**, **4**, **5**, and **13**–**21**.

Compound[a]		C-1	C-2	C-3	C-4	C-5	C-6
1	A	100.1	69.6	68.4	65.7	69.4	62.2
	B	97.6	69.2	69.0	65.7	68.4	62.1
	C	97.3	78.8	80.0	72.8	74.5	67.1
4	A	97.0	79.9	81.1	64.8	74.1	66.1
	B	100.5	69.2	69.2	65.5	69.4	62.6
	C	97.6	69.3	69.0	66.3	68.4	62.2
5	A	97.3	78.8	80.0	72.8	74.5	67.1
	B	100.5	69.4	68.6	65.8	69.5	62.2
	C	99.0	69.4	68.3	66.2	69.7	62.7
	D	97.8	69.2	68.9	66.0	68.6	62.1
13	A	98.4	69.4	68.9	65.9[b]	69.2	65.8
	B	97.2	70.6	74.8	65.8[b]	69.3	66.6
	C	99.0	69.8	68.3	65.9[b]	68.6	62.0
	D	97.4	69.2	68.0	65.6	69.3	62.3
14	A	98.1	71.2	75.2	68.0	70.2	62.5
	B	99.0	70.8	74.7	67.4	69.6	66.2
	C	98.8	69.6	68.3	65.5	69.3	62.2[b]
	D	97.5	69.1	69.1	65.9	68.4	61.9[b]
15	A	98.3	71.1	75.0	n. d.	n. d.	66.2[b]
	B	99.1	70.8	75.0	n. d.	n. d.	66.0
	C	97.2	70.6	75.4	n. d.	n. d.	66.6[b]
	D	98.9[b]	69.8	n. d.	n. d.	n. d.	62.1
	E	99.1[b]	69.8	n. d.	n. d.	n. d.	62.1
	F	97.6	69.2	n. d.	n. d.	n. d.	62.3
	G	97.6	69.0	n. d.	n. d.	n. d.	62.3
16	A	98.3	71.1	74.6	68.2	69.4	66.7
	B	99.1[b]	70.8	75.0	67.4	69.8	66.3
	C	97.3	70.2	69.9	66.6	68.8	62.7
	D	98.9[b]	69.6	68.4	65.6	69.4	62.0
	E	97.5	69.1[c]	69.2[c]	66.0	68.5	62.3
17	A	98.6	70.8	74.2	68.1	69.5	66.3
	B	98.7	70.8	69.2	66.6	69.6	62.8
	C	97.4	70.8	74.9	68.1	69.5	66.7
	D	99.1	69.9	68.5	65.8	69.4	62.1
	E	97.5	69.4	69.1	66.0	68.7	62.4
18	A	98.4	70.8	73.9	68.9	69.5	66.8
	B	98.7	70.7	69.2	66.6	69.6	62.9
	C	97.6	69.5	69.3	65.6	69.7	65.7
	D	97.4	70.7	75.0	68.1	69.5	67.2
	E	99.1	69.8	68.5	65.8[b]	69.4	62.1
	F	97.6	69.4	69.1	65.9[b]	68.7	62.4
19	A	98.2	70.9	75.4	68.1	69.3	66.8
	B	99.3	69.9	68.6	65.6	70.2	65.9
	C	97.3	70.3	69.9	66.7	68.9	62.8
	D	97.6	70.5	74.9	68.1	69.3	66.8
	E	98.8	69.9	68.6	65.9[b]	69.3	61.9
	F	97.6	69.3	69.0	65.6[b]	68.6	62.3
20	A	98.2	70.9	75.3	68.4	69.4	67.1
	B	99.3	69.9	68.5	65.6	70.2	66.0
	C	97.7	69.7	69.3	65.7	69.7	65.6
	D	97.7	70.7[b]	75.0	68.2	69.4	66.8
	E	97.5	70.6[b]	75.0	68.1	69.4	66.8
	F	99.1[c]	69.9	68.6	66.0[d]	69.3	62.0[e]
	G	98.9[c]	69.9	68.6	66.0[d]	69.3	62.1[e]
	H	97.7	69.4	69.1	65.8[d]	69.1	62.4
	I	97.6	69.4	69.1	65.8[d]	69.1	62.4
21	A	81.3	72.4	75.4	68.5	69.9	66.9
	B	99.1	69.9	68.5	65.5	70.3	66.1
	C	97.6	69.4	69.0[b]	65.6	69.6	65.5
	D	97.7	70.5	74.9	68.1	69.4	66.8
	E	97.5	70.6	74.9	68.0	69.4	66.8
	F	98.8	69.8	68.5	65.9[c]	69.4	62.1[d]
	G	99.1	69.7	68.5	65.9[c]	69.4	62.0[d]
	H	97.6	69.4	69.0[b]	65.7[c]	68.6	62.4[d]
	I	97.6	69.4	69.2[b]	65.7[c]	68.6	62.4[d]

[a] The rows correspond to individual α-D-mannopyranose residues designated as **A**, **B**, **C**, etc. (see schemes). [b,c,d,e] Assignment within the group of signals may be interchanged. n. d. = not determined.

Table 4. Chemical shifts (δ values) of the mannopyranosidic protons in the ^1H NMR spectra (D_2O, 25°C) of deprotected mannooligosaccharides **22**–**29**.

Compound[a]		H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
22	A	4.76	3.94	3.93	3.74	3.75	3.79	3.95
	B	4.88	4.14	3.91	3.89	3.77	3.78	3.97
	C	5.13	4.06	3.87	3.67	3.78	3.76	3.86
	D	4.91	3.98	3.84	3.66	3.70	3.78	3.89
23	A	4.73	4.09	3.83	3.90	3.75	3.75	3.97
	B	5.07	4.23	4.01	3.86	3.96	3.76	3.98
	C	4.87	4.14	3.93	3.87	3.88	3.78	3.98
	D	5.13	4.07	3.89	3.66	3.79	3.75	3.90
	E	5.15	4.07	3.89	3.67	3.77	3.77	3.90
	F	4.89	3.98	3.83	3.67	3.68	3.77	3.88
	G	4.91	3.98	3.83	3.67	3.68	3.77	3.88
24	A	4.80	4.05	3.78	3.86	3.75	3.69	3.98
	B	5.21	4.33	4.08	3.94	4.04	3.81	4.05
	C	4.97	4.06	3.91	3.73	3.76	3.84	3.96
	D	5.25	4.15	3.96	3.72	3.87	3.87	3.96
	E	4.98	4.07	3.92	3.74	3.76	3.84	3.96
25	A	4.72	4.07	3.83	3.88[b]	3.78	3.74	3.97
	B	5.09	4.06	3.87	3.64	3.76	3.75	3.86
	C	4.87	4.12	3.91	3.90[b]	3.86	3.78	3.95
	D	5.13	4.05	3.87	3.64	3.76	3.75	3.85
	E	4.90	3.96	3.82	3.64	3.67	3.75	3.89
26	A	4.72	4.07	3.85	3.85	3.77	3.75	3.95
	B	5.10	4.06	3.86	3.67	3.75	3.76	3.87
	C	4.89	3.99	3.82	3.71	3.82	3.79	3.91
	D	4.87	4.12	3.98	3.85	3.87	3.78	3.94
	E	5.11	4.06	3.86	3.67	3.77	3.76	3.87
	F	4.90	3.98	3.82	3.67	3.67	3.76	3.87
27	A	4.78	4.07	3.83	3.85	3.77	3.77	3.97
	B	5.07	4.06	3.87	3.72	3.93	3.79	3.93
	C	4.92	3.98	3.83	3.68	3.70	3.78	3.89
	D	4.85	4.13	3.87	3.88	3.87	3.80	3.96
	E	5.11	4.06	3.88	3.68	3.78	3.78	3.90
	F	4.92	3.98	3.83	3.68	3.70	3.78	3.89
28	A	4.79	4.08	3.83	3.83	3.77	3.77	3.96
	B	5.08	4.08	3.88	3.72	3.93	3.79	3.93
	C	4.91	4.00	3.84	3.73	3.83	3.83	3.95
	D	4.87	4.13	3.88	3.88	3.95	3.81	4.00
	E	4.89	4.12	3.91	3.88	3.95	3.80	3.97
	F	5.12	4.07	3.89	3.68	3.77	3.77	3.90
	G	5.13	4.07	3.88	3.69	3.77	3.77	3.90
	H	4.92	3.98	3.83	3.68	3.68	3.81	3.95
	I	4.92	3.98	3.84	3.68	3.68	3.81	3.95
29	A	5.44	4.23	3.89	3.95	4.28	3.79	4.03
	B	5.13	4.13	3.93	3.74	3.97	3.83	3.98
	C	4.94	4.03	3.88	3.77	3.86	3.86	3.94
	D	4.91	4.22	4.01	3.93	3.93	3.84	4.03
	E	4.94	4.18	3.98	3.94	3.92	3.83	4.03
	F	5.24	4.12	3.94	3.73	3.82	3.82	3.96
	G	5.18	4.12	3.94	3.73	3.83	3.83	3.93
	H	4.97	4.04	3.89	3.72	3.76	3.82	3.94
	I	4.97	4.04	3.89	3.72	3.76	3.82	3.94

[a] The rows correspond to individual α-D-mannopyranose residues designated as **A**, **B**, **C**, etc. (see schemes). [b] Assignment within the group of signals may be interchanged

(10.1 g) with a mobility like that of the trisaccharide derivative **4**, and this fraction was acetylated. Crystallization from MeOH gave the trisaccharide cyanoethylidene derivative **1** (7.2 g, 46%). Column chromatography of the mother liquor afforded **5** (3.3 g; 16%) as an amorphous solid, $[\alpha]_D = +41$ ($c = 2.9$); elemental analysis calcd (%) for $C_{51}H_{67}NO_{33}$ (1221.1): C 50.12, H 5.53, N 1.15; found: C 50.37, H 5.67, N 1.05.

Methyl 2,3,4-tri-*O*-acetyl-6-*O*-[2,4-di-*O*-acetyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranoside (13): A solution of methyl 2,3,4-tri-*O*-acetyl-6-*O*-trityl-α-D-mannopyranoside (**6**) (0.145 g, 0.26 mmol) and the cyanoethylidene derivative **1** (0.241 g,

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Table 5. Chemical shifts (δ values) of the mannopyranosidic carbons in the ^{13}C NMR spectra (D_2O, 25 °C) of deprotected mannooligosaccharides **22**–**29**.

Compound[a]		C-1	C-2	C-3	C-4	C-5	C-6
22	A	100.7	69.6	70.7	66.3	70.5[b]	65.1[c]
	B	100.9	70.9	79.8	67.2	71.0[b]	67.1[c]
	C	103.5	71.5	71.8	68.2	74.6	62.4
	D	100.7	71.4	72.0	68.2	74.1	62.4
23	A	102.1	70.8	80.0	66.7	71.9	67.0
	B	103.5	70.8	79.5	67.0	73.0	66.5
	C	100.5	70.8	79.5	67.0	72.1	66.6
	D	103.4	71.2	71.6	68.0	74.5	62.1
	E	103.3	71.2	71.6	68.0	74.4	62.1
	F	100.8	71.2	71.8	68.0	73.8	62.3
	G	100.5	71.2	71.8	68.0	73.8	62.1
24	A	101.8	70.8	80.1	66.6	72.0	66.3
	B	103.3	70.8	79.6	67.0	73.0	66.8
	C	100.4	71.2	71.9	68.0	73.9	62.1
	D	103.2	71.2	71.5	68.0	74.6	62.1
	E	100.2	71.2	71.8	67.9	73.8	62.3
25	A	101.9	71.2	80.2	67.5	72.6	67.0
	B	103.2	71.7	72.2	68.4	74.9	62.6
	C	100.3	71.2	80.0	67.4	72.5	67.0
	D	103.1	71.7	72.0	68.4	74.9	62.6
	E	100.2	71.6	72.0	68.4	74.3	62.6
26	A	102.2	70.9	79.8	67.2	72.4	66.9
	B	103.4	71.4	71.8	68.2	74.6	62.3
	C	100.7	71.4	72.0	68.2	72.5	67.1
	D	100.6	70.9	79.8	67.1	72.4	66.7
	E	103.4	71.4	71.8	68.2	74.6	62.3
	F	100.7	71.4	72.0	68.2	74.0	62.3
27	A	102.2	71.3	81.0	66.7	72.0	66.4
	B	104.1	71.0	71.9	68.0	73.0	68.0
	C	100.5	71.3	71.9	68.0	74.0	62.3
	D	101.1	70.8	79.9	67.0	72.0	66.4
	E	103.7	71.3	71.9	68.0	74.7	62.3
	F	100.7	71.3	71.9	68.0	74.0	62.3
28	A	102.2	71.2	80.8	67.1	72.0	66.9[b]
	B	103.8	71.5	73.1	68.3	72.3	67.9
	C	100.8	71.5	72.5	68.3	72.3	67.4[b]
	D	101.2	71.0	80.0	67.4	72.1	67.0[b]
	E	100.8	71.0	79.9	67.4	72.1	67.0[b]
	F	103.5	71.5	72.1	68.3	74.7	62.5
	G	103.5	71.5	72.1	68.3	74.7	62.5
	H	100.8	71.5	72.0	68.3	74.1	62.5
	I	100.8	71.5	72.0	68.3	74.1	62.5
29	A	86.3	73.9	81.4	67.4	72.6	66.7
	B	104.2	71.5	72.4	68.3	73.5	67.3
	C	100.8	71.5	72.5	68.3	72.4	67.3
	D	101.5	71.1	80.0	67.3	72.0	66.8
	E	100.8	71.1	80.1	67.3	72.0	66.8
	F	103.8	71.6	72.4	68.3	74.9	62.5
	G	103.9	71.6	72.4	68.3	74.9	62.5
	H	100.8	71.5	72.1	68.3	74.2	62.5
	I	100.8	71.5	72.1	68.3	74.2	62.5

[a] The rows correspond to individual α-D-mannopyranose residues designated as **A**, **B**, **C**, etc. (see schemes). [b,c] Assignment within the group of signals may be interchanged.

0.26 mmol) in PhH (2 mL) was placed in one limb of a tuning fork-shaped tube,[15] and a solution of $TrClO_4$ (0.009 g, 0.026 mmol) in dry $MeNO_2$ (0.2 mL) was placed in the other. The tube was connected to a vacuum line ($(3-4)\times10^{-3}$ Torr) and the solutions were freeze-dried. PhH (2 mL) was then distilled into the limb containing the reagents, the resulting solution was freeze-dried once more, and the residue was dried at 30–40 °C for about 30 min. CH_2Cl_2 (2 mL) was then distilled into the tube, the solutions of the reagents and the catalyst were mixed, and the mixture was left overnight at about 20 °C. A drop of C_5H_5N was then added to the bright yellow solution, whereupon it became colorless. The solution was diluted with CH_2Cl_2, washed with water, and concentrated. Column chromatography (PhH/EtOAc, 1:1) afforded the tetrasaccharide **13** (0.171 g, 56%); $[\alpha]_D=+47.9$ ($c=0.86$); elemental analysis calcd (%) for $C_{51}H_{70}O_{34}$ (1227.0): C 49.92, H 5.75; found: C 49.89, H 5.76.

Methyl 2,4-di-*O*-acetyl-3-*O*-[2,4-di-*O*-acetyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-6-*O*-trityl-α-D-mannopyranoside (14): Glycosylation of methyl 2,4-di-*O*-acetyl-3,6-di-*O*-trityl-α-D-mannopyranoside (**7**; 0.199 g, 0.260 mmol) with **1** (0.244 g, 0.260 mmol) in the presence of $TrClO_4$ (8.9 mg, 0.026 mmol) in CH_2Cl_2 (2 mL), as described for the preparation of **13**, followed by column chromatography (PhMe/EtOAc, 1:2) afforded the tetraoside **15** (0.202 g, 55%); $[\alpha]_D=+35.9$ ($c=1.7$); elemental analysis calcd (%) for $C_{68}H_{82}O_{32}$ (1410.6): C 57.90, H 5.86; found: C 57.90, H 5.55.

Methyl 2,4-di-*O*-acetyl-3,6-di-*O*-[2,4-di-*O*-acetyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranoside (15): Glycosylation of **7** (0.114 g, 0.150 mmol) with **1** (0.280 g, 0.300 mmol) in the presence of $TrClO_4$ (10 mg, 0.03 mmol) in CH_2Cl_2 (2 mL), as described above, followed by column chromatography (PhMe/EtOAc, 1:2) afforded the heptaoside **15** (0.195 g; 59%); $[\alpha]_D=+50.3$ ($c=1.4$); elemental analysis calcd (%) for $C_{87}H_{118}O_{58}$ (2091): C 49.93, H 5.59; found: C 50.09, H 5.80; MALDI-TOF MS: *m/z*: 2117 and 2133 (calcd for $[M+Na]^+$ and $[M+K]^+$ *m/z*: 2114 and 2130, respectively).

Methyl 2,4-di-*O*-acetyl-3-*O*-[2,4-di-*O*-acetyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-α-D-mannopyranoside (16): A mixture of the trityl ether **14** (0.42 g, 0.23 mmol), AgOTf (0.23 g, 0.89 mmol), and 2,4,6-collidine (0.01 mL, 0.09 mmol) in PhH was freeze-dried. A solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (0.583 g, 0.89 mmol; pre-dried by lyophilization of PhH) in CH_2Cl_2 (5 mL) was added dropwise with stirring at 0 °C under argon to a solution of the above mixture in CH_2Cl_2 (5 mL). A bright yellow coloration typical of the triphenylmethylium cation appeared immediately. After 10 min, a drop of C_5H_5N was added (discoloration was observed), the solution was diluted with $CHCl_3$, and the precipitate was filtered off. The filtrate was washed with 1M aqueous $Na_2S_2O_3$ and water, and then concentrated. Column chromatography of the residue (PhH/EtOAc, 1:1) gave the pentaoside **16** (0.244 g; 47%); $[\alpha]_D=+20.3$ ($c=2.2$); elemental analysis calcd (%) for $C_{83}H_{94}O_{42}$ (1763.6): C 56.53, H 5.37; found: C 56.20, H 5.45.

Methyl 2,4-di-*O*-acetyl-6-*O*-[2,4-di-*O*-acetyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-α-D-mannopyranoside (17): Glycosylation of methyl 2,4-di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-6-*O*-trityl-α-D-mannopyranoside (**8**; 0.30 g, 0.27 mmol) with **1** (0.257 g, 0.27 mmol) in CH_2Cl_2 (2 mL) in the presence of $TrClO_4$ (9 mg, 0.027 mmol), as described above, followed by column chromatography (PhH/EtOAc, 1:1), afforded the pentaoside **17** (0.315 g; 66%); $[\alpha]_D=+21.2$ ($c=2.4$); elemental analysis calcd (%) for $C_{83}H_{94}O_{42}$ (1763.6): C 56.53, H 5.37; found: C 56.19, H 5.48; FAB MS: *m/z*: 1785 $[M+Na-H]^+$.

Methyl 2,4-di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-6-*O*-{2,3,4-tri-*O*-acetyl-6-*O*-[2,4-di-*O*-acetyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranosyl}-α-D-mannopyranoside (18): Glycosylation of methyl 2,4-di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-6-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-trityl-α-D-mannopyranosyl)-α-D-mannopyranoside (**9**; 0.175 g, 0.126 mmol) with compound **1** (0.117 g, 0.126 mmol) in CH_2Cl_2 (2 mL) in the presence of $TrClO_4$ (4 mg, 0.0126 mmol), as described above, followed by column chromatography (PhH/EtOAc, 1:1), afforded the hexaoside **18** (0.150 g; 58%); $[\alpha]_D=+87.1$ ($c=1.0$); $C_{95}H_{110}O_{50}$ (2051.9): elemental analysis calcd C 55.61, H 5.40; found: C 55.45, H 5.36; FAB MS: *m/z*: 2074 $[M+Na-H]^+$.

Methyl 2,4-di-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-3-*O*-{2,3,4-tri-*O*-acetyl-6-*O*-[2,4-di-*O*-acetyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranosyl}-α-D-mannopyranoside (19): The hexaoside **19** was synthesized by condensation of methyl 2,4-di-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-3-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-trityl-α-D-mannopyranosyl)-α-D-mannopyranoside (**10**; 0.257 g, 0.198 mmol) with **1** (0.185 g, 0.198 mmol) in CH_2Cl_2 (2 mL) in the presence of $TrClO_4$ (6.8 mg, 0.02 mmol), as described above. Column chromatography (PhH/EtOAc,

1:3) yielded **19** (0.20 g; 60%); $[\alpha]_D = +29.4$ ($c = 2.4$); elemental analysis calcd (%) for $C_{95}H_{110}O_{50}$ (2051.9): C 55.61, H 5.40; found: C 55.45, H 5.36.

Methyl 2,4-di-*O*-acetyl-3,6-di-*O*-{2,3,4-tri-*O*-acetyl-6-*O*-[2,4-di-*O*-acetyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranosyl}-α-D-mannopyranoside (20): The nonaoside **20** was synthesized by the condensation of methyl 2,4-di-*O*-acetyl-3,6-di-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-trityl-α-D-mannopyranosyl)-α-D-mannopyranoside (**11**; 0.201 g, 0.15 mmol) with the cyanoethylidene derivative **1** (0.280 g, 0.3 mmol) in CH_2Cl_2 (2 mL) in the presence of $TrClO_4$ (10 mg, 0.03 mmol), as described above. Column chromatography (PhH/EtOAc, 1:3) yielded **20** (0.20 g; 50%); $[\alpha]_D = +54.3$ ($c = 2.4$); elemental analysis calcd (%) for $C_{111}H_{150}O_{74}$ (2668.4): C 49.96, H 5.67; found: C 50.17, H 5.86; FAB MS: *m/z*: 2690 $[M+Na-H]^+$.

Ethyl 2,4-di-*O*-acetyl-3,6-di-*O*-{2,3,4-tri-*O*-acetyl-6-*O*-[2,4-di-*O*-acetyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranosyl}-1-thio-α-D-mannopyranoside (21): Glycosylation of ethyl 2,4-di-*O*-acetyl-3,6-di-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-trityl-α-D-mannopyranosyl)-1-thio-α-D-mannopyranoside (**12**; 0.548 g, 0.4 mmol) was performed with the cyanoethylidene derivative **1** (0.821 g, 0.88 mmol) in the presence of $TrClO_4$ (27 mg, 0.08 mmol) in CH_2Cl_2 (7–8 mL), as described above. Conventional work-up and column chromatography (PhH/EtOAc, 1:4 → 1:19) afforded the nonaoside **21**; yield 0.58 g (54%); $[\alpha]_D = +65.1$ ($c = 2.4$); $C_{112}H_{152}O_{73}S$ (2698.4); FAB MS: found: *m/z*: 2721 $[M+Na]^+$.

Methyl 6-*O*-(3,6-di-*O*-α-D-mannopyranosyl-α-D-mannopyranosyl)-α-D-mannopyranoside (22): Methanolic NaOMe solution (0.5 M, 0.1 mL) was added to a solution of the acetate **13** (120 mg) in MeOH (2 mL) and C_5H_5N (1 mL), and the reaction mixture was allowed to stand overnight at about 20 °C. It was then neutralized with cation-exchange resin KU-2 (H^+) that had been pre-washed with MeOH, the resin was filtered off, and the filtrate was concentrated to give the methyl tetraoside **22** in virtually quantitative yield.

Table 6. Optical rotations for oligosaccharides **22**–**29**.

Oligosaccharide	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**
$[\alpha]_D$	+70.3	+127.2	+95.7	+87.5	+31.9	+31.4	+74.9	+98.0
(c, H_2O)	(2.3)	(0.4)	(1.3)	(0.4)	(1.7)	(0.8)	(0.8)	(1.4)

Deprotected oligosaccharides 23–29: These compounds were prepared from the corresponding acylated derivatives **15**–**21**. In a typical experiment, 0.5 M methanolic NaOMe (0.1 mL) was added to a solution of compound **19** (100 mg) in a mixture of dry MeOH (1 mL) and dry C_5H_5N (1 mL), and the solution was left overnight at about 20 °C. It was then diluted with H_2O and the mixture was neutralized with cation-exchange resin KU-2 (H^+). The resin was filtered off, methyl benzoate was extracted with PhMe, and the aqueous solution was concentrated to dryness to give the hexaoside **27** in virtually quantitative yield. The optical rotation values of the free oligosaccharides thus obtained are listed in Table 6. Literature values for compound **24**: $[\alpha]_D = +98.3$ (H_2O);[35b] compound **25**: $[\alpha]_D = +108.1$ (H_2O).[35a]

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