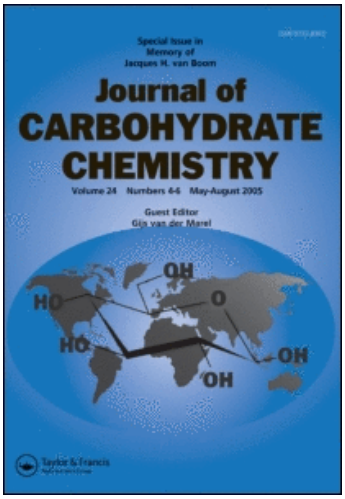

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SYNTHESIS AND NMR CHARACTERIZATION OF SOPHOROSYL TREHALOSE TETRASACCHARIDES

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ABSTRACT

Glycosidation of 2',3',6'-tri-*O*-benzyl-α-D-glucopyranosyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**2**) with α-aceto-bromosophorose (**1**) gave the α- and β-linked tetrasaccharides **3** and **4** in an approximately 2:1 ratio in dichloromethane or acetonitrile. The reaction is discussed, notably the predominant formation of α-glycosides. Both compounds were fully characterized by ^{1}H and ^{13}C NMR spectroscopy applying 1D TOCSY, 1D T-ROESY, ^{1}H-detected one-bond and multiple bond $^{1}H,^{13}C$ 2D COSY. Deprotection of **3** and **4** furnished the free sophorosyl trehaloses **7** and **8**.

INTRODUCTION

The pseudo-nonasaccharide derivative Trestatin A sulfate has been identified[1] to be highly active in the inhibition of smooth muscle cell growth which is a pivotal process in the development of arteriosclerotic lesions.[2] Unlike the polysaccharide heparin, Trestatin A sulfate exhibited no antithrombin III mediated anticoagulant properties. To allow the investigation of compounds of even lower molecular weight, Trestatin A tri-, tetra-, and pentasaccharide substructures have been prepared, namely (1→4)-linked glucosyl,[3] maltosyl, and maltotriosyl[4] trehaloses. Due to the biological properties of these oligosaccharides[5] the preparation of analogues became of

interest. We describe here the synthesis and characterization of two isomeric sophorosyl trehalose tetrasaccharides.

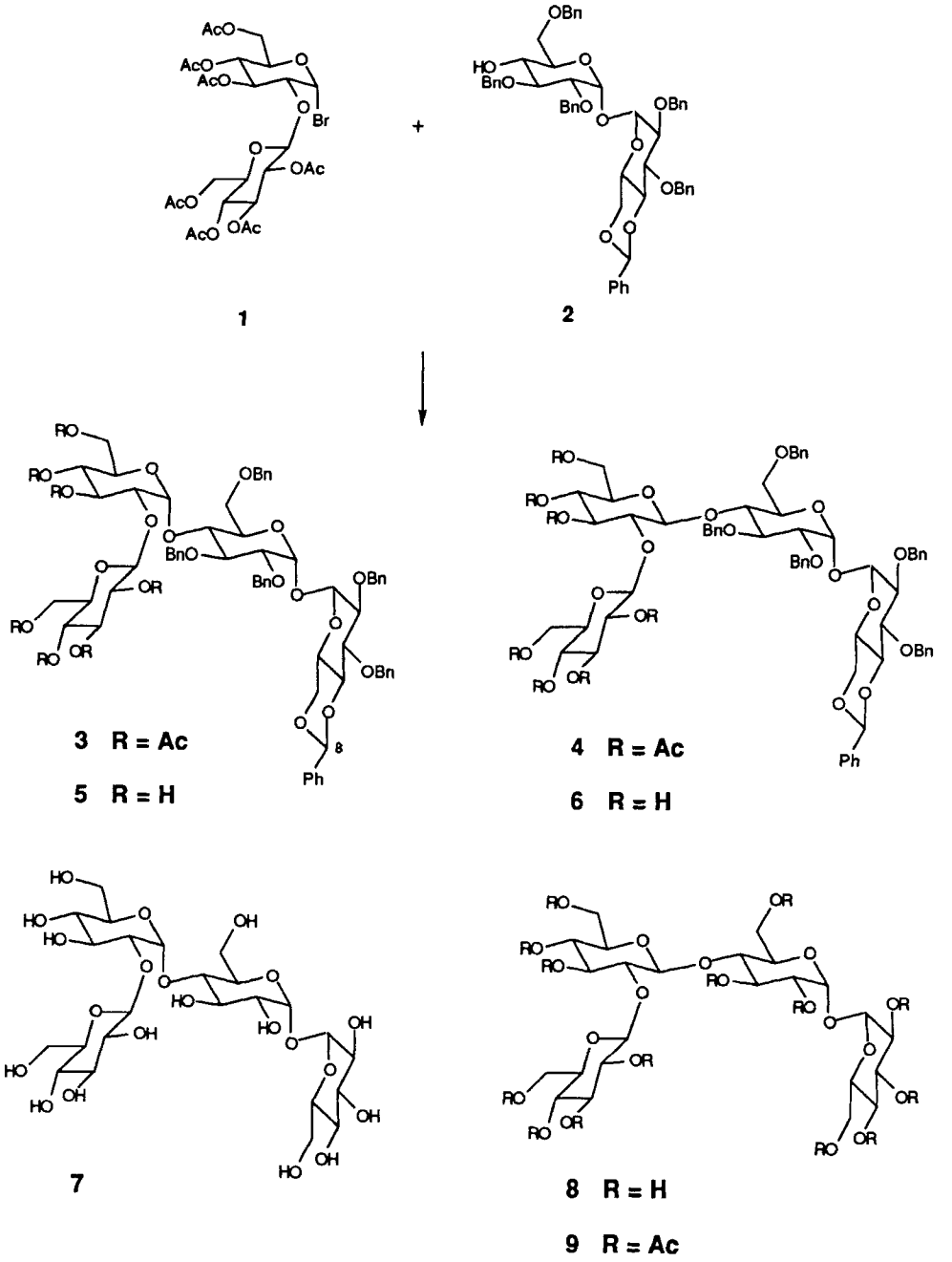
RESULTS AND DISCUSSION

Chemistry. For the precursors of (1→4)-linked sophorose (2-*O*-β-D-glucopyranosyl-D-glucose) and α,α-trehalose (α-D-glucopyranosyl α-D-glucopyranoside), we chose α-aceto-bromosophorose (**1**)[6] as glycosyl donor and 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl 2',3',6'-tri-*O*-benzyl-α-D-glucopyranoside (**2**) as well established glycosyl acceptor.[3] For the synthesis of bromide **1** we followed the work of Pfander and collaborators;[7] a modification in the work-up using a filtration through silica gel instead of crystallization could increase the yield of bromide to 95 %.

Glycosidation of acceptor **2** with sophorosyl bromide **1** in dichloromethane using silver oxide on Celite and drierite as an activating agent afforded α-sophoroside **3** in 14.2 % yield after column chromatography along with a mixed fraction of **3** and **4** (34.8 %, α/β = 2:1) as well as pure isolated β-sophoroside **4** (9.5 %). Acetonitrile as solvent gave a comparable result to that of dichloromethane; tetrahydrofuran as a solvent led to lower yields. Low yields, but also a preference for the formation of α-sophoroside, were obtained with silver zeolite[8] in dichloromethane (4.2 % **3**, 1.5 % **4**). Surprisingly, even lower conversion was observed with silver triflate and tetramethylurea[9] in dichloromethane.

An activated sophorose is an interesting glycosyl donor in the sense that it carries a non-participating neighbouring group next to the anomeric center. So far, only few syntheses of sophorosyl glycosides have been described in literature, and the stereochemical outcome of the glycosidations was not uniform. Reaction of sophorosyl bromide **1** with benzyl alcohol in the presence of mercuric cyanide resulted in the formation of 76 % of benzyl β-sophoroside.[10] Likewise, in the glycosidation of the phenolic hydroxyl groups of flavanones naringenin and hesperetin, β-sophorosides were obtained using silver carbonate/drierite in 7 % and 14 % yield, respectively;[11] similar results were found in the glycosidation of glycyrrhetic acid (4 % β-sophoroside, silver carbonate and iodine).[12] Phase transfer catalysis conditions furnished better yields of a flavanoid sophoroside (45 %), also in this example only β-glycosidation was reported.[13] Sophorosyl transfer to the primary hydroxyl group of glucose afforded mainly the β-isomer (49 % β;

SCHEME 1



silver oxide on Celite, iodine),[7] corresponding to the coupling of **1** to the primary hydroxyl group of a glycerol derivative which gave the β-sophoroside as major product (27.5 % β, 16 % α; mercuric oxide and bromide, iodine, drierite as catalysts).[14] In contrast, glycosidation of the tertiary hydroxyl group of 1-methylcyclohexanol with **1** furnished α-sophorosides exclusively with different catalysts, the more complex steviol methyl ester also afforded mainly α-product (86 %) along with some β-sophoroside (3.1 %).[15] Although these results on the sophorosidation of various glycosyl acceptors with different catalyst systems can not be compared in a straightforward manner, it is obvious that only the unreactive tertiary hydroxyl groups as acceptors led to α-glycosides. Thus, it seems that the reactivity of the glycosyl acceptor determines the stereochemistry of the glycosidation. Conversely, the steric demand of the (1→2)-linked glucosyl moiety of sophorose was postulated by Pfander and collaborators[7] to direct the stereochemistry. Our findings on the predominant formation of α-sophoroside **3** is in keeping with above statement since the low reactivity of the trehalose glycosyl acceptor **2** is well known.[2,3]

Acetonitrile as a solvent has been shown to exert a distinct effect on the course of glycosidation reactions with a non-participating protecting group at C-2 via the formation of intermediate nitrilium glycosides.[16,17] In the sophorosidation reaction discussed above we could not observe any β-directing influence of acetonitrile whatsoever. These findings break the rule of consistent β-selectivity in the presence of acetonitrile as a solvent.[18] This is probably due to reaction conditions (elevated temperature, long reaction time) necessary for the unreactive glycosyl acceptor which favours the formation of the thermodynamically more stable β-nitrilium intermediate.

Finally, both tetrasaccharides **3** and **4** were deprotected by standard procedures. Deacetylation furnished **5** and **6**, and hydrogenation gave the deblocked tetrasaccharides **7** and **8**. Since the signal for H-1" in the ^{1}H NMR spectrum of **8** was obscured by the HDO peak, this compound was further characterized by its peracetate **9**.

NMR Investigations. Both tetrasaccharides **3** and **4** were characterized by NMR spectroscopy, and the ^{1}H and ^{13}C assignments of the carbohydrate part are given in the experimental part. As was shown previously,[3,4,19] the 1D TOCSY (or HOHAHA) experiment is a very efficient tool for extracting the ^{1}H NMR subspectra of the different carbohydrate moieties in strongly overlapping carbohydrate spectra. In this experiment, a selective 180° pulse,

provided here by a DANTE pulse train, at first inverts the magnetization of a selected proton the signal of which should be sufficiently separated from those of its neighbours. After an unselective 90° pulse, followed by an MLEV-17 mixing sequence[19] of suitable duration, the magnetization of the excited proton is relayed, step by step, to its spin-coupled neighbouring protons. From the difference FID (on-resonance minus off-resonance excitation) the subspectrum of the selected carbohydrate moiety is obtained with excellent resolution as in a normal 1D spectrum.

The assignment of the different protons is readily derived from the successive appearance of their signals when the mixing time is increased in steps.[3] A sequence of four or five 1D TOCSY experiments with mixing times between about 30 and 360 ms is, in general, sufficient for this purpose. If preferred, only one experiment with a long mixing time can be supplemented by a 2D COSY with a much lower digital resolution, sufficient, however, to reveal the sequence of coupled protons.

Thus, in three sets of 1D TOCSY experiments with **3**, inversion of the magnetization of the isolated signals of H-1'', H-1''', and H-6a' led to the identification of the subspectra of the sophorosyl pyranoses and of one of the trehalose rings. Mixing times of 30, 60, 120, 240, and 360 ms were used in these experiments. Excitation of the two exactly equivalent protons H-1 and H-1' then led to the identification of the protons of the remaining trehalose ring except H-6. The assignment of the obtained subspectra to the different rings is eventually readily achieved by measuring the NOE that detects close spatial approach between protons of different rings. Additional information was derived from the long-range $^{1}H,^{13}C$ COSY.

It is well known that the longitudinal NOE is less suited in this range of molecular masses since it is expected to be close to zero.[20] In agreement with this we have observed only a few extremely weak positive NOEs in some steady state 1D NOE difference experiments with **3**. On the other hand, the application of its variant, called transverse or rotating frame NOE or ROE,[20,21] revealed a number of typical strong through-space contacts that were used to assign the subspectra to the different rings.

In contrast to our previous 1D (and 2D) ROESY experiments[4] that used a train of 90° pulses (the compensated ROESY protocol[22]) for spin-locking we have found here and at other occasions[23] that the T-ROESY spin-locking scheme[24] gave better results virtually free of TOCSY artefacts. Thus, 1D T-ROESY experiments could unequivocally discriminate both trehalose rings

showing ROEs of medium or strong intensity between H-8_{ax} and H-4_{ax}/H-6_{ax}, and between H-1'' and H-4', along with H-2'' and, in low intensity, H-3''. The α-sophorosyl linkage was evident from the shift and coupling constant of H-1'' ($J_{1'',2''}$ = 3.7 Hz).

Based on the proton assignment achieved in this way the corresponding assignment of the ^{13}C NMR signals was in most cases readily accessible by interpretation of the one-bond and multiple-bond $^{1}H,^{13}C$ 2D COSY acquired in reverse mode. Particularly the long-range version provided relevant additional structural information such as the confirmation of the linkage of the rings and the assignment of the attached side groups. However, since several proton shifts as well as some carbon shifts were very close to each other, there still remained a number of ambiguities. As an example, since H-1 and H-1' had exactly the same shifts and no suitable long-range cross peaks were detected or resolved in the $^{1}H,^{13}C$ COSY, C-1 and C-1' could not be distinguished in the beginning.

A recently proposed combination of the 1D TOCSY and INEPT sequences[25] was used to resolve several of the remaining ambiguities. This experiment is in the first part fully analogous to the 1D TOCSY sequence described before. In the second part the relayed proton magnetizations are now further transferred by an INEPT sequence to their directly bonded carbons. The longer the mixing time, the more ^{13}C signals of the selected ring become visible and carbons up to seven bonds away from the excited proton have been detected.[25] It is important to note that the carbon directly attached to the excited proton can not be detected since only the proton of the corresponding ^{12}C isotopomer is selectively excited.

This experiment hence allows to identify by a simple 1D experiment ^{13}C signals belonging to a selected carbohydrate ring. However, it has the inherent disadvantage that selective irradiation at the chemical shift of a given proton of a ^{12}C isotopomer may accidentally perturb one of the two ^{13}C side bands of other proton signals that absorb $J_{CH}/2$ Hz away from the signal in the ^{12}C isotopomers. This can lead to the appearance of additional ^{13}C signals that may belong to a different ring. An example will be discussed below. A further important disadvantage of this experiment is its low sensitivity caused by the fact that the much weaker ^{13}C signal is detected in natural abundance of only 1 %. Thus relatively high concentrations are needed to obtain reasonable spectra in an acceptable acquisition time (see Experimental).

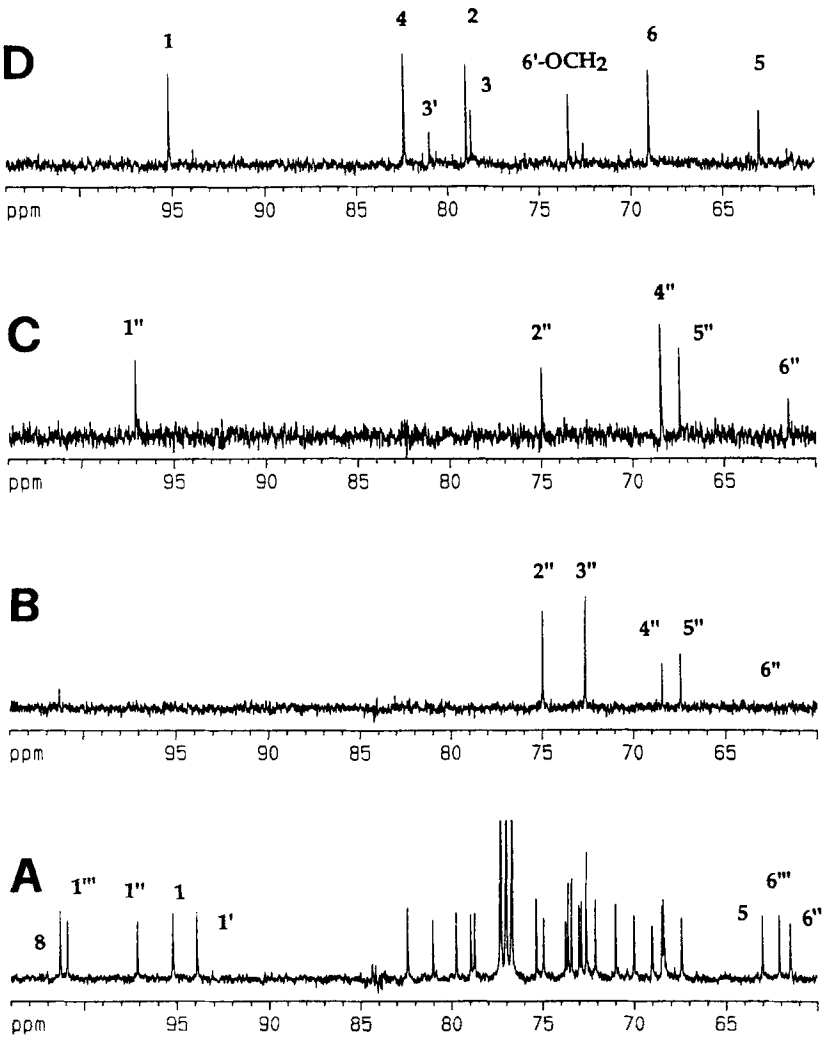


FIG. 1. Part of the ^{13}C NMR (100.6 MHz, in CDCl3) of **3** (trace A) and three examples of 1D TOCSY-INEPT difference spectra (B, C and D) that generate ^{13}C subspectra of individual carbohydrate rings. After selective inversion by a 180° DANTE pulse sequence of the magnetization of a selected proton, followed by an MLEV-17 mixing sequence, magnetization is transferred to other neighbouring coupled protons in the same coupling network. The following INEPT sequence transfers this magnetization to the directly attached ^{13}C nuclei. Only their signals are observed in the difference spectrum. In experiment B proton H-1" at 5.88 ppm was excited and a mixing time of 80 ms was applied. In C, H-3" at 5.47 ppm (160 ms), and in D the overlapping signals of H-3 and H-5 at 4.27 ppm were selectively inverted (40 ms).

In Figure 1, three 1D TOCSY-INEPT experiments (B, C and D) with **3** are presented as examples together with the relevant part of the normal ^{1}H decoupled ^{13}C NMR (A). In B, the magnetization of proton H-1" at 5.88 ppm was selectively inverted by a 180° DANTE pulse train, followed by an unselective 90° pulse and an MLEV-17 mixing sequence of 80 ms duration. This transfers magnetization to H-2", H-3", H-4", H-5", and very weakly, to H-6". The subsequent INEPT sequence transfers this proton magnetization to the corresponding attached ^{13}C nuclei, therefore, only these signals appear in the difference spectrum. In experiment C, proton H-3" was excited and after a mixing time of 160 ms all carbons except C-3" are seen in the spectrum. Experiments B and C thus help to distinguish between the very closely spaced signals of C-4" at 68.23 ppm and C-4"' at 68.51 ppm. In experiment D, H-3 and H-5, both absorbing at ca. 4.27 ppm, were excited. All the ^{13}C signals of this ring are now identified. Here, the ^{13}C signals of C-3 and C-5 are now visible since magnetization was exchanged between both protons via H-4. In this spectrum, a number of additional undesired signals are observed that are quite obviously caused by an insufficient selectivity of the excitation as was discussed before. Despite this drawback, the distinction between C-1 and C-1' is now unambiguously possible.

Tetrasaccharide **4** was characterized in an analogous way. Excitations of the anomeric protons in 1D-TOCSY experiments provided the complete assignment of the ^{1}H NMR spectrum. In a T-ROESY experiment, through-space contacts were observed between H-1" and H-4'/H-3' proving the identity of both pyranosides involved. In addition, both glucopyranosyl moieties of trehalose can be distinguished by the two large coupling constants of H-5 ($J_{4,5}$ = 9.9 Hz, $J_{5,6b} \approx$ 10 Hz), since H-6 is locked in one conformation by the benzylidene ring. In particular, the typical coupling constant of H-1" ($J_{1",2"}$ = 7.7 Hz) shows that **4** is the β-sophorosyl analogue of **3**.

EXPERIMENTAL

General Procedures. Solvents and reagents were obtained from Fluka. Evaporation: *in vacuo*, conducted with Büchi rotary evaporator. TLC: precoated silica gel 60F-254 plates (Merck), detection by UV light (254 nm) and spraying with a 10% solution of concentrated sulfuric acid in methanol followed by heating. Specific rotations: Perkin-Elmer Polarimeter 241. MS: PE API III (Sciex, Toronto). NMR: Bruker AM-400 (400 MHz for ^{1}H, 100.6 MHz

for ^{13}C) with Aspect 3000 computer and 160 Mbyte disk; chemical shifts in ppm relative to tetramethylsilane or sodium 2,2,3,3-tetradeutero-3-(trimethylsilyl)-propionate as internal standard.

NMR techniques. 1D TOCSY difference spectra were measured with the decoupler as source of proton excitation as recently described in detail.[3] Typical parameters were as follows: DANTE pulse sequence 680 times 2 μs with a delay between pulses of 70 μs at decoupler power level of 18H; MLEV-17 mixing times of 30, 60, 120, 240, and 360 μs; 10 * 16 scans with 10 z-filter delays between 5 and 50 ms. A typical total acquisition time was ca. 60 min.

T-ROESY experiments were performed as described previously[23] with $(180_x\text{-}180_{-x})_n$ spin-lock of 0.6 s duration (n=2400). A typical acquisition time was ca. 60 min.

The ^1H, ^{13}C 2D COSY were measured in the reverse mode with the decoupler as source for proton excitation. The one-bond experiment was tuned to a one-bond coupling of 139 Hz; the long-range version was tuned to 7 Hz. Typically, 350 experiments were acquired with 16 scans in the one-bond case (total duration ca. 3h, 20 mg) and 64 scans in the long-range case (ca. 11h).

The 1D TOCSY-INEPT pulse sequence was essentially as proposed in ref. 25. Since no shaped soft pulse was available on our AM-400 spectrometer, a 180° DANTE pulse train was used as in 1D TOCSY for selective excitation with typical settings of 1050 times 2 μs duration with an interpulse delay of 70 μs at a decoupler power setting of 17H. The MLEV-17 sequence, that was flanked by two trim pulses of 2.5 ms, lasted as specified in the legend of Figure 1. The delays Δ1 and Δ2 in the INEPT sequence were set to 1.56 and 1.06 ms.[25] The difference FID was acquired between on-resonance and off-resonance selective excitation. The experiments shown in Figure 1 needed 5h acquisition time (6000 scans, relaxation delay 2s, 75 mg of sample) to obtain a reasonable signal-to-noise using a dual probe head with higher sensitivity for ^{13}C.

***O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide (1).** A solution of octa-*O*-acetyl-sophorose (6.85 g, 10.1 mmol) in dry dichloromethane (15 mL) was treated with 35 % HBr in acetic acid at 0-5 °C. After 2 h the reaction mixture was poured into ice/water and extracted with dichloromethane. The extracts were washed with cold water, dried over $MgSO_4$, and concentrated. The residue was filtered over coarse silica gel (90 g) using dichloromethane/diethyl ether as solvent. Evaporation of solvents furnished pure **1** (6.74 g, 95 %): mp 189-190 °C, ref. 7: mp 189-190

°C; ref. 6: mp 194 °C, decomp; $[\alpha]_D^{20}$ +93.8° (*c* 0.5, chloroform), ref. 7: $[\alpha]_D^{20}$ +95.1° (*c* 1, chloroform), ref. 6: $[\alpha]_D^{20}$ +95.6° (chloroform).

***O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (3) and *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (4).** A solution of glycosyl acceptor **2** (17.6 g, 20 mmol) in dichloromethane (100 mL) was stirred for 2 h in the presence of silver oxide on Celite[7] (10.0 g) and pulverized molecular sieves (15 g) under argon and exclusion of light. Then iodine (0.50 g) was added, followed by a solution of bromide **1** (6.74 g, 9.64 mmol). The reaction mixture was heated to reflux for 65 h, cooled, and filtered. The filter cake was carefully washed with dichloromethane. The organic solvents of the filtrates were evaporated. The residue was chromatographed on silica gel (350 g) using ether/dichloromethane 1:4 as eluent to remove unreacted glycosyl acceptor. Product fractions were pooled and rechromatographed on silica gel (350 g) with ethyl acetate/hexane 1:2, 2:3, and 1:1 to obtain pure tetrasaccharide **4** (1.37 g, 9.5 %) followed by a fraction of **3** and **4** (5.02 g, 34.8 %) and pure tetrasaccharide **3** (2.05 g, 14.2 %).

3: Syrup; $[\alpha]_D^{20}$ +70.2° (*c* 0.5, chloroform); MS (ionspray): 1517 (80 %, $[M+NH_4]^+$); ^1H NMR (400 MHz, $CDCl_3$; 1D TOCSY, 1D T-ROESY) δ 7.54-7.52 (m, 2 H, aromatic), 7.43-7.09 (m, 28 H, aromatic), 5.88 (d, 1H, $J_{1'',2''}$ = 3.7 Hz, H-1''), 5.56 (s, 1H, H-8), 5.47 (dd ~ t, 1H, $J_{3'',4''}$ = 9.5 Hz, H-3''), 5.13 (d, 2H, H-1, H-1'), 5.05 (dd ~ t, 1H, H-3'''), 5.05, 4.97 (2d, 2H, J = 11.0 Hz, 3-CH_2Ph), 5.01 (s, 2H, 3'-CH_2Ph), 4.99 (dd ~ t, 1H, H-4''), 4.94 (dd, 1H, $J_{2''',3'''}$ = 9.7 Hz, H-2'''), 4.91, 4.65 (2d, 2H, J = 12.0 Hz, 2-CH_2Ph), 4.76 (dd ~ t, 1H, $J_{3''',4'''}$ = 9.2 Hz, H-4'''), 4.69 (d, 1H, $J_{1''',2'''}$ = 8.0 Hz, H-1'''), 4.63, 4.57 (2d, 2H, J = 11.5 Hz, 2'-CH_2Ph), 4.43, 4.39 (2d, 2H, J = 12.0 Hz, 6'-CH_2Ph), 4.27 (dd ~ t, 1H, $J_{3,4}$ = 9.0 Hz, H-3; ddd ~ dt, 1H, $J_{5,6a}$ = 4.7 Hz, H-5), 4.13 (ddd ~ br d, 1H, H-5'), 4.12 (dd, 1H, $J_{5'',6a''}$ = 2.9 Hz, $J_{6a'',6b''}$ = 12.0 Hz, H-6a''), 4.11 (dd, 1H, H-6a), 4.10 (dd ~ t, 1 H, $J_{3',4'}$ = 8.8 Hz, H-3'), 3.98 (dd, 1H, $J_{4',5'}$ = 10.0 Hz, H-4'), 3.91 (dd, 1H, $J_{2'',3''}$ = 10.0 Hz, H-2''), 3.89 (ddd ~ dt, 1H, $J_{4'',5''}$ = 10.0 Hz, $J_{5'',6b''}$ =2.1 Hz, H-5''), 3.75 (dd, 1H, $J_{5''',6a'''}$ = 2.0 Hz, $J_{6a''',6b'''}$ = 11.8 Hz, H-6a'''), 3.68 (dd, 1H, H-6b''), 3.67 (dd ~ t, 1H, $J_{6a,6b}$ ≈ 10.5 Hz, H-6b), 3.65 (dd ~ t, 1H, $J_{4,5}$ ≈ 10 Hz, H-4), 3.62 (dd, 1H, $J_{1,2}$ = 3.8 Hz, $J_{2,3}$ = 9.4 Hz, H-2), 3.57 (dd, 1H, $J_{1',2'}$ = 3.8 Hz, $J_{2',3'}$ = 9.6 Hz, H-

2'), 3.55 (dd, 1H, H-6b'''), 3.51 (ddd, 1 H, $J_{4''',5'''}$ = 9.9 Hz, $J_{5''',6b'''}$ = 5.8 Hz, H-5'''), 3.35 (dd, 1H, $J_{5',6a'}$ = 3.0 Hz, $J_{6a',6b'}$ = 11.5 Hz, H-6a'), 3.19 (dd, 1H, $J_{5',6b'}$ = 1.6 Hz), 2.11, 2.07, 2.00, 1.97, 1.91, 1.87, 1.86 (7s, 21H, OAc); ^{13}C NMR (100.6 MHz, $CDCl_3$; DEPT-135, one-bond and long-range $^1H,^{13}C$ COSY) δ 170.47 (2), 170.32, 169.98, 169.84, 169.20, 168.67 (7 CO), 139.10, 139.07, 138.37, 137.96, 137.76, 137.58 (C_{ar}), 128.85-126.22 (C_{ar}), 101.31 (C-8), 100.91 (C-1'''), 97.09 (C-1''), 95.18 (C-1), 93.89 (C-1'), 82.40 (C-4), 81.03 (C-3'), 79.74 (C-2'), 78.96 (C-2), 78.74 (C-3), 75.36 (C-Ph), 74.95 (C-2''), 73.75 (3'-C-Ph), 73.61 (C-Ph), 73.42 (C-Ph), 73.00 (C-Ph), 72.87 (C-3'''), 72.60 (C-3'' and C-4'), 72.11 (C-5'''), 71.02 (C-2'''), 70.02 (C-5'), 69.03 (C-6), 68.51 (C-4'''), 68.43 (C-4''), 68.38 (C-6'), 67.42 (C-5''), 63.02 (C-5), 62.07 (C-6'''), 61.48 (C-6''), 21.04, 20.69 (2), 20.58, 20.53 (2), 20.24, (7 Ac).

4: Syrup; $[\alpha]_D^{20}$ +39.0° (*c* 0.5, chloroform); MS (ionspray): 1517 (70 %, $[M+NH_4]^+$); ^1H NMR (400 MHz, $CDCl_3$; 1D TOCSY, 1D T-ROESY) δ 7.50-7.47 (m, 2 H, aromatic), 7.43-7.21 (m, ca. 28 H, aromatic), 5.52 (s, 1H, H-8), 5.15 (d, 1H, $J_{1',2'}$ = 3.8 Hz, H-1'), 5.11 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1), 5.08 (dd ~ t, 1H, $J_{3''',4'''}$ = 9.4 Hz, H-3'''), 5.03, 4.91 (2d, 2H, J = 11.0 Hz, CH_2Ph), 4.91 (dd ~ t, 1H, H-3''), 4.91, 4.80 (2d, 2 H, J = 10.8 Hz, CH_2Ph), 4.90, 4.73 (2d, 2H, CH_2Ph), 4.88 (dd ~ t, 1H, H-4''), 4.86 (dd, 1H, $J_{2''',3'''}$ = 9.5 Hz, H-2'''), 4.74 (dd ~ t, 1H, $J_{4''',5'''}$ = 9.0 Hz, H-4'''), 4.71, 4.43 (2d, 2H, J = 12.0 Hz, CH_2Ph), 4.70, 4.65 (2d, 2H, J = 11.5 Hz, CH_2Ph), 4.53 (d, 1H, $J_{1''',2'''}$ = 8.0 Hz, H-1'''), 4.36 (d, 1H, $J_{1'',2''}$ = 7.7 Hz, H-1''), 4.28 (ddd ~ dt, 1H, $J_{4,5}$ = 9.9 Hz, H-5), 4.17 (dd, 1H, $J_{5''',6a'''}$ = 5.8 Hz, $J_{6a''',6b'''}$ = 12.1 Hz, H-6a'''), 4.14 (dd, 1H, H-6a''), 4.13 (dd and br d, 2H, H-5', H-6b'''), 4.11 (dd ~ t, 1H, $J_{2,3} \approx 8.4$ Hz, H-3), 4.06 (dd, 1H, $J_{5,6a}$ = 5.0 Hz, $J_{6a,6b}$ = 10.1 Hz, H-6a), 3.99 (dd, 1H, $J_{5',6a'}$ = 2.2 Hz, $J_{6a',6b'}$ = 11.0 Hz, H-6a'), 3.92 (m_c, 2H, H-3', H-4'), 3.84 (dd, 1H, $J_{5'',6b''}$ = 2.2 Hz, $J_{6a'',6b''}$ = 12.2 Hz, H-6b''), 3.63 (d, 1H, $J_{5',6b'} \leq 1$ Hz, H-6b'), 3.62 (dd ~ t, 1H, $J_{5,6b} \approx 10$ Hz, H-6b), 3.61 (dd ~ t, 1H, $J_{3,4}$ = 9.8 Hz, H-4; dd, 1H, H-2''), 3.60 (dd, 1H, H-2), 3.56 (dd, 1H, H-2'; ddd, 1H, $J_{5''',6b'''}$ = 5.0 Hz, H-5'''), 3.12 (ddd, 1H, $J_{5'',6a''}$ = 4.0 Hz, $J_{4'',5''}$ = 9.0 Hz, H-5''), 2.08, 2.06, 1.98, 1.96, 1.94, 1.93, 1.92 (7s, 21H, Ac); ^{13}C NMR (100.6 MHz, $CDCl_3$, DEPT-135, ^1H-detected one-bond and multiple bond $^1H,^{13}C$ 2D COSY) δ 170.48, 170.14, 169.99, 169.82, 169,67 (5 CO), 169.2 (2 CO), 139.30, 138.75, 138.13, 138.01 137.43 (5 C_{ar}), 128.68-125.93 (Car), 100.96 (C-8), 100.52 (C-1'''), 100.29 (C-1''), 95.54 (C-1), 94.69 (C-1'), 82.32 (C-4), 80.10 (C-3'), 78.68 (C-3), 78.51 (C-2), 78.20 (C-2'), 77.7 (C-2''), 77.20 (C-4'), 75.01 (C-Ph), 74.92 (C-3''), 74.78, 73.68, 73.30, 73.05 (4 C-Ph), 72.63 (C-3'''), 71.93 (C-5'''), 70.9 (C-5''), 70.88 (C-5''), 70.75 (C-2'''), 70.54 (5'), 69.25 (C-4'''), 68.86 (C-6), 68.14 (C-4''), 67.65 (C-6'), 62.68 (C-6'''), 62.53 (C-5), 61.56 (C-6''), 20.75, 20.66, 20.49 (3 Ac), 20.41 (2 Ac), 20.37, 20.26 (2 Ac).

O-β-D-Glucopyranosyl-(1→2)-O-α-D-glucopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranosyl 2,3-di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside (5). A solution of tetrasaccharide **3** (1.95 g, 1.3 mmol) in diethyl ether (4 mL) and methanol (20 mL) was kept at room temperature in the presence of sodium methanolate (3.9 mL 1 % solution in methanol) for 8 h. Then the reaction mixture was neutralized with Amberlite IR120 (H^+) and filtered. The filtrate and methanol washings were taken to dryness. The residue was chromatographed using ethyl acetate/methanol/water 96:2:2 as eluent to furnish pure **5** (1.29 g, 83 %) as a syrup, $[\alpha]_D^{20}$ +82.8° (*c* 0.5, chloroform); MS (ionspray): 1222.8 (40 %, $[M+NH_4]^+$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.62 (br s, 1H, H-1''), 5.51 (s, 1H, CHPh), 5.10 (br s, 1H, H-1' or H-1), 5.05 (br s, 1H, H-1 or H-1').

O-β-D-Glucopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranosyl 2,3-di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside (6). Tetrasaccharide **4** (1.28 g, 0.85 mmol) was deacetylated as described above for the preparation of **5** to give pure **6** (0.92 g, 90 %) as a syrup after chromatography, $[\alpha]_D^{20}$ +52.8° (*c* 0.5, chloroform); MS (ionspray): 1222.8 (100 %, $[M+NH_4]^+$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.50 (s, 1H, CHPh), 5.11 (d, 1H, $J_{1',2'}$ = 3.6 Hz, H-1' or H-1), 5.08 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1 or H-1'), 4.73 (d, 1H, $J_{1'',2''}$ = 3.3 Hz, H-1''), 4.37 (d, 1H, $J_{1''',2'''}$ = 7.1 Hz, H-1''').

O-β-D-Glucopyranosyl-(1→2)-O-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl α-D-glucopyranoside (7). A solution of **5** (1.25 g, 1.03 mmol) in ethanol (36 mL) and water (12 mL) was hydrogenated in the presence of 10 % palladium-on-carbon (0.8 g) at room temperature for 8 h. The reaction mixture was filtered, and the residue was washed with ethanol/water. The filtrates were taken to dryness to give **7** as a syrup quantitatively, $[\alpha]_D^{20}$ +133.2° (*c* 0.5, water); MS (ionspray): 689.3 (100 %, $[M+Na]^+$); ^1H NMR (400 MHz, D_2O) δ 5.69 (d, 1H, $J_{1'',2''}$ = 3.7 Hz, H-1''), 5.18-5.17 (m, 2H, H-1, H-1'), 4.66 (d, 1H, $J_{1''',2'''}$ = 7.9 Hz, H-1''').

O-β-D-Glucopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl α-D-gluco-pyranoside (8). Tetrasaccharide **6** (1.64 g, 1.36 mmol) was hydrogenated as described above for **7** to give pure **8** quantitatively as a syrup, $[\alpha]_D^{20}$ +81° (*c* 0.1, water); MS (ionspray): 689.3 (100 %, $[M+Na]^+$), 667.2 (35 %, $[M+H]^+$); ^1H NMR (400 MHz, D_2O) δ 5.18-5.17 (m, 2H, H-1, H-1'), 4.62 (d, 1H, $J_{1''',2'''}$ = 7.7 Hz, H-1''').

O-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-(1→2)-O-(3,4,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-α-D-glucopyranosyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (9). A solution of **8** (89 mg, 0.13 mmol) in pyridine (1.8 mL) and acetic anhydride (0.9 mL) was kept for 1 day and then reduced in volume by evaporation. The residue was taken up in ethyl acetate and washed with cold dilute aqueous sulfuric acid, brine, saturated bicarbonate solution, and brine. The organic phase was dried over magnesium sulfate, concentrated, and chromatographed over silica gel (ethyl acetate/hexane 1:1 containing 4 % methanol) to give **9** (121 mg, 72 %) as a syrup, $[\alpha]_D^{20}$ +71.5° (*c* 0.2, chloroform); MS (ionspray): 1272.3 (100 %, $[M+NH_4]^+$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.27 (d, 1H, $J_{1',2'}$ = 3.9 Hz, H-1' or H-1), 5.18 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1 or H-1'), 4.68 (d, 1H, J = 8.1 Hz, H-1''' or H-1''), 4.47 (d, 1H, J = 7.5 Hz, H-1'' or H-1'''), 2.149, 2.146, 2.142, 2.11, 2.10, 2.07, 2.06 (7s, 7x3H, Ac), 2.04 (s, 6H, Ac).

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