Synthesis of C-Oligosaccharides That Mimic Their Natural O-Analogous Immunodeterminants in Binding to Monoclonal Immunoglobulins

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We have stereoselectively synthesized the analogues of the methyl β-glycosides of (1→6)-β-D-*galacto*-oligosaccharides (up to tetrasaccharide), in which the interglycosidic oxygen atoms are replaced by a methylene group.

Introduction

C-Oligosaccharides are close analogues of oligosaccharides whose intersaccharidic oxygen bridges have been replaced by methylene groups. Such compounds are impervious to hydrolytic enzymes and as such are potentially of great use as pharmacodynamic agents. A basic question then is to evaluate whether such a structural modification − which eradicates the *exo*-anomeric effect − modifies the conformational properties of the molecule, resulting in enhanced or impaired biological activity. For this reason, various strategies for the basic construction of C-disaccharides have recently been developed,[1] followed by studies of their conformation, either free in solution[2] or protein-bound.[3]

Less attention has so far been devoted to a direct evaluation of the biological activity of these carbohydrate mimics. The group of Kishi has demonstrated that the C-trisaccharide analogue of the H-type II human blood group antigenic determinant is still recognized by the lectin UEA-I[4] with the same affinity. The binding of C-lactose to the lectin ricin B[3a] and to the enzyme β-galactosidase[3b] has been studied. Recently, we have shown[5] that a mixed synthetic C,O-pentasaccharide displays an anti-factor Xa activity similar to that of the corresponding O-pentasaccharide of heparin/heparan sulfate. This augurs well for the future of these mimics, and it is evident that the continued examination of the biochemical behaviour of O- and C-analogue pairs is important.

The group of Glaudemans has extensively studied the binding of β(1→6)-D-galactan onto various monoclonal immunoglobulins.[6] It has been shown that these proteins bind best to a tetrasaccharidic epitope. The specific strategy that we have used to synthesize a β(1→6)-C-disaccharide[1a] − indeed the first preparation of a member of this class of carbohydrate mimics − appeared to us adaptable enough to the preparation of β(1→6)-C-D-galactans to launch such a synthetic programme.

Results and Discussion

The three targets are the C-oligosaccharides **8, 14,** and **20** (Figure 1), the C-analogues of oligosaccharides already synthesized.[7] The use of oligomers in which the downstream terminal sugar residue has been converted into the methyl glycoside allows the interpretation of binding data to be free of the ambiguities that would otherwise result from ring equilibrium at the reducing end. This also simplifies the interpretation of structural NMR data. The β-methyl galactoside was logically selected to mimic the natural configuration of the β(1→6)-D-galactan.

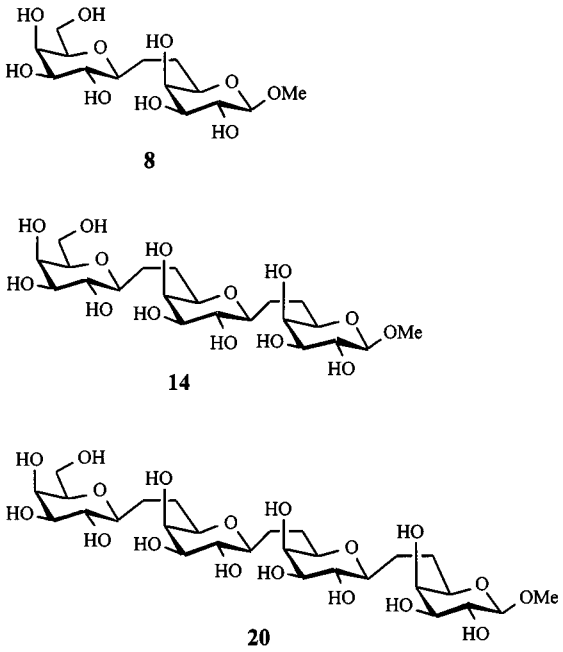

Figure 1. Target compounds

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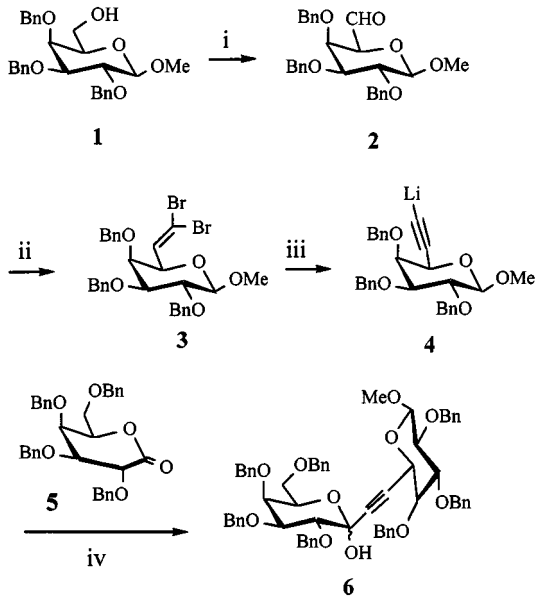
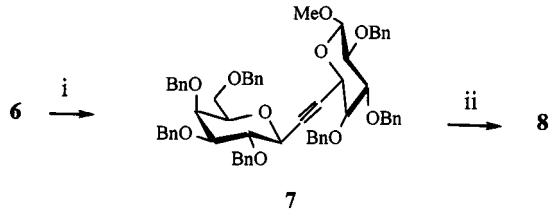
Synthesis of the C-Disaccharide 8

This compound was prepared (Scheme 1) with the method previously developed by us for the expeditious preparation of a β(1→6)-C-disaccharide.[1a] The known[8] methyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside **1** was oxidized by pyridinium chlorochromate (PCC) in dichloromethane in the presence of 4 Å molecular sieves to the corresponding C-6 aldehyde **2**,[9] which was purified by chromatography. Treatment with carbon tetrabromide and triphenylphosphane in dichloromethane gave the dibromo olefin **3** in crystalline form. The acetylenic anion **4**, derived in situ from treatment of **3** by *n*-butyllithium in THF at −78°C, was allowed to react with known[10] 2,3,4,6-tetra-*O*-benzyl-D-galactopyranolactone **5** to give the hemiacetal **6** in 75% yield.



Scheme 1. Reagents: i) PCC, CH_2Cl_2, 84%; ii) CBr_4, Ph_3P, CH_2Cl_2, 87%; iii) *n*BuLi (2 equiv.), THF, −78°C → −20°C; iv) THF, −78°C → room temp., 75%

As shown in Scheme 2, stereospecific reduction of **6** with triethylsilane in dichloromethane, in the presence of BF_3−diethyl ether, gave the protected crystalline β-C-disaccharide **7** in 82% yield, as the only isolated product. Hydrogenation/hydrogenolysis of **7** (H_2, Pd/C, 2:1, MeOH−AcOEt) gave crystalline methyl 6-deoxy-6-*C*-(2,6-anhydro-1-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl)-β-D-galactopyranoside **8**.

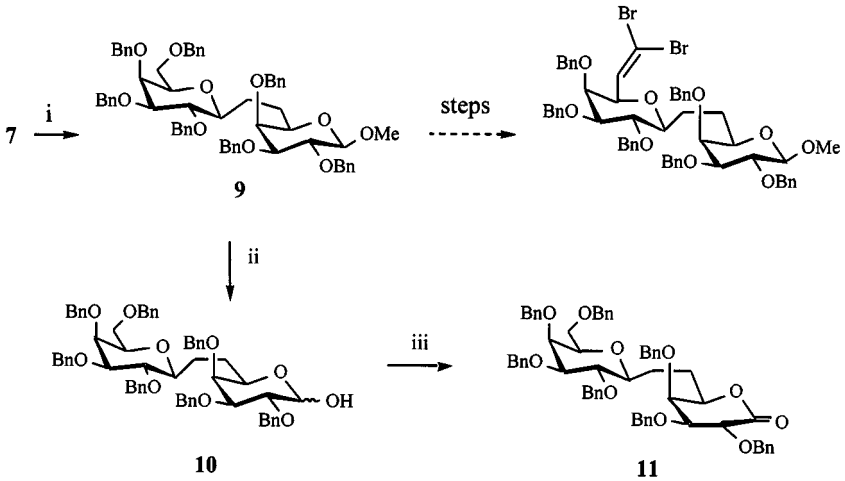


Scheme 2. Reagents: i) Et_3SiH, $BF_3 \times OEt_2$, CH_2Cl_2/CH_3CN, 82%; ii) H_2, Pd/C, MeOH, AcOEt, 90%

Synthesis of the C-Trisaccharide 14

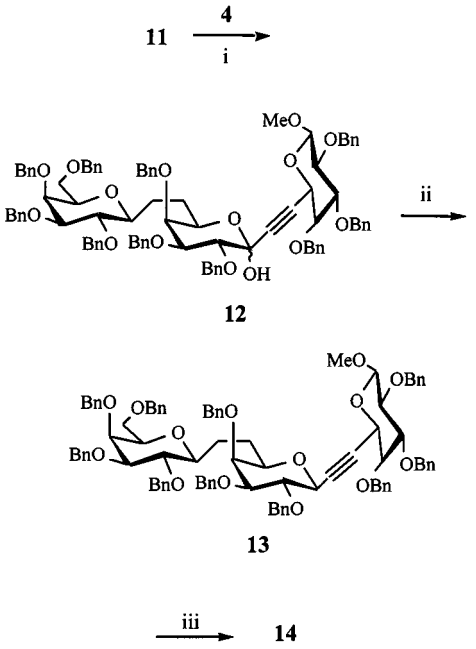
In order to apply the same methodology to the synthesis of the C-trisaccharide, the C-disaccharide **7** was first selectively hydrogenated to **9** (H_2/PtO_2, AcOEt). Two options are then possible: either the conversion of **7** into a disaccharide lactone **11** through the hemiacetal **10** or into a disaccharidic dibromo olefin (Scheme 3).

A success in doing the two transformations would simultaneously open a way to a direct synthesis of a C-tetrasaccharide by block synthesis (2 + 2). The selective removal through acetolysis[11] of the primary benzyl ether of **9** led to a mixture of products and this transformation was abandoned. The selected option was thus a downstream elongation. Acid hydrolysis of **9** (H_2SO_4, AcOH, 60°C, 15 h) gave the hemiacetal **10** in 71%, which was uneventfully (87% yield) oxidized to the corresponding lactone **11** by pyridinium chlorochromate (PCC) in dichloromethane in the presence of 4 Å molecular sieves. As shown in the self-



Scheme 3. Reagents: i) H_2, PtO_2, AcOEt, 93%; ii) H_2SO_4, AcOH, 60°C, 15 h, 71%; iii) PCC, 4-Å molecular sieves, CH_2Cl_2, 87%

explanatory Scheme 4, reiteration of the previous sequence (Scheme 1) gave the C-trisaccharide **14**.



Scheme 4. Reagents: i) THF, −78°C → room temp., 67%; ii) Et_3SiH, $BF_3 \times OEt_2$, CH_2Cl_2/CH_3CN, 82%; iii) H_2, Pd/C, MeOH/AcOEt, 90%

Synthesis of the C-Tetrasaccharide 20

Following the downstream methodology previously used for the synthesis of C-trisaccharide **14**, compound **13** was converted into C-tetrasaccharide **20**, as shown in the self-explanatory Scheme 5.

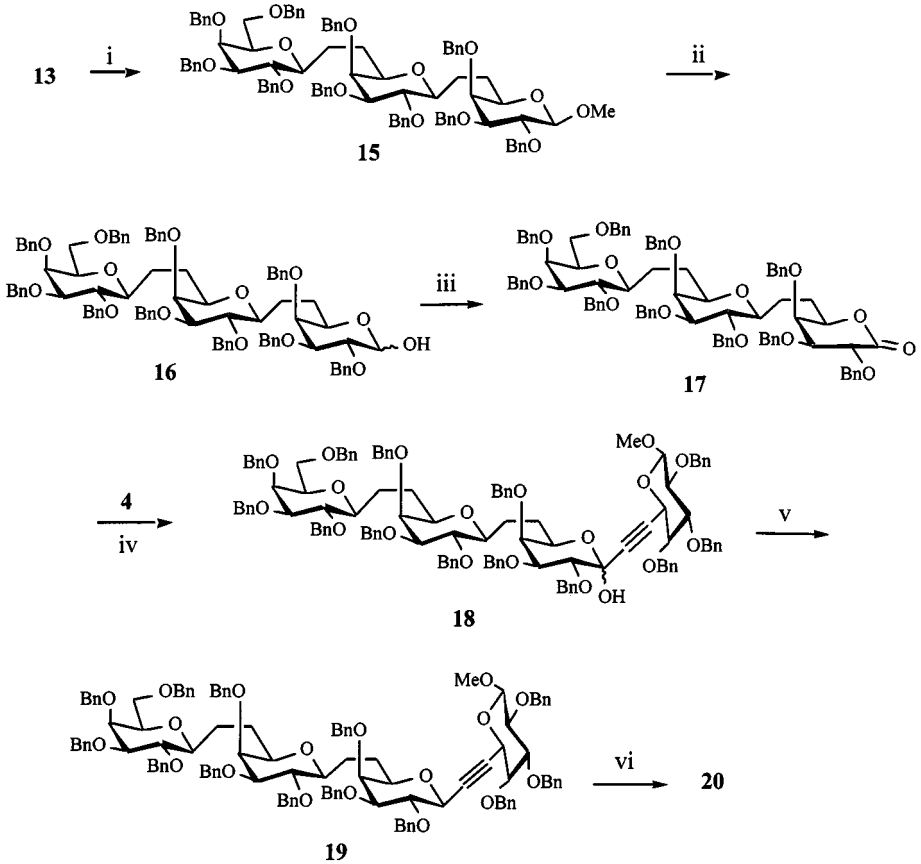
Binding Studies and Conclusion

The free energy of association of each of the three C-oligosaccharides **8**, **14** and **20** with three different monoclonal immunoglobulins, whose specificity is for sequential *O*-β(1→6)-D-galactopyranosyl determinants, has been measured by the group of Glaudemans and has been reported elsewhere.[12] The conclusion was that the C-analogues behave the same in binding to the monoclonal antibodies as do the natural *O*-linked immunodeterminants.

A study of carbohydrate-protein interaction includes examination of hydrogen bonding between carbohydrate determinants and protein epitopes with deoxy sugars as probe. In the present study, the replacement of the interglycosidic oxygen atoms by methylene groups negates the participation of these oxygen atoms in a hydrogen bond important for the binding.

Experimental Section

General: Melting points (m.p.) were determined with a Büchi 510 apparatus and are uncorrected. − Optical rotations were measured



Scheme 5. Reagents: i) H_2, PtO_2, AcOEt, 96%; ii) H_2SO_4, AcOH, 60°C, 15 h, 51%; iii) PCC, 4-Å molecular sieves, CH_2Cl_2, 88%; iv) THF, −78°C → room temp., 68%; v) Et_3SiH, $BF_3 \times OEt_2$, CH_2Cl_2/CH_3CN, 78%; vi) H_2, Pd/C, MeOH/AcOEt, 91%

at 20 ± 2°C with a Perkin Elmer 241 digital polarimeter. − CI (ammonia) mass spectra were taken with a Nermag R10-10 spectrometer. − Elemental analyses were performed by Service Central d'Analyse du CNRS, BP 22, 69390 Vernaison, France or Service d'Analyse de l'Université Pierre et Marie Curie, F-75252 Paris Cedex 05, France. − ^{1}H-NMR spectra were determined with Bruker AM 200, AM-250, and AM-400 spectrometers using Me_4Si as internal standard. − ^{13}C-NMR spectra were determined with Bruker AM-250 (62 MHz) and AM-400 (100.57 MHz) spectrometers using Me_4Si as reference (δ = 0). − Reactions were monitored by TLC on silica gel 60 F_{254} (Merck) and detection by charring with sulfuric acid. − Flash column chromatography was performed on silica gel 60 (230−400 mesh, Merck).

Methyl 2,3,4-Tri-*O*-benzyl-β-D-*galacto*-hexodialdo-1,5-pyranoside (2): A mixture of methyl 2,3,4-*O*-benzyl-β-D-galactopyranoside (**1**) (8.7 g, 18.7 mmol), pyridinium chlorochromate (PCC, 4.4 g, 20 mmol) and 4-Å molecular sieves in dry dichloromethane (200 mL) was stirred for 2 h at room temp., filtered through Celite, and concentrated. The residue was chromatographed (cyclohexane/AcOEt, 3:1) to give the corresponding aldehyde **2** (7.3 g, 84%). − ^{1}H NMR (250 MHz, $CDCl_3$): δ = 9.61 (d, 1 H, *J* = 0.5 Hz), 7.40 (m, 15 H), 5.00−4.50 (m, 6 H), 4.35 (d, 1 H, *J* = 7.6 Hz), 4.20 (dd, 1 H, *J* = 2.9 Hz, *J* = 0.6 Hz), 3.88 (dd, 1 H, *J* = 9.6 Hz, *J* = 7.6 Hz), 3.72 (dd, 1 H, *J* = 0.6 Hz, *J* = 0.5 Hz), 3.61 (s, 3 H), 3.55 (dd, *J* = 9.6 Hz, *J* = 2.96 Hz).− MS (CI; NH_4^+); *m/z* (%): 480 (100) [M + 18].

Methyl 2,3,4-Tri-*O*-benzyl-7,7-dibromo-6,7-dideoxy-β-D-*galacto*-hept-6-ynopyranoside (3): A solution of tetrabromomethane (10.5 g, 31.5 mmol) in dry dichloromethane (80 mL) was added to a solution of triphenylphosphane (16.5 g, 63.2 mmol) in dry dichloromethane (150 mL) at 0°C. The mixture was stirred for 5 min, then a solution of **2** (7.29 g, 15.8 mmol) in dry dichloromethane (100 mL) was added and the resulting mixture was stirred for 2 h. Water (200 mL) and dichloromethane were added. The organic layer was dried ($MgSO_4$) and concentrated. The residue was chromatographed (cyclohexane/AcOEt, 6:1) to afford **3** (8.48g, 87%), m.p. 75−76°C (cyclohexane, AcOEt). − $[\alpha]_D$ = −43 (*c* = 1.0, chloroform). − ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.42−7.25 (m, 15 H), 6.62 (d, 1 H, *J* = 7.3 Hz), 4.95−4.64 (m, 6 H), 4.28 (d, 1 H, *J* = 7.7 Hz), 3.96 (d, 1 H, *J* = 7.3 Hz, *J* < 0.5 Hz), 3.80 (m, 2 H, *J* = 7.7 Hz, *J* = 10.0 Hz, *J* = 9 Hz, *J* < 0.5 Hz), 3.55 (s, 3 H), 3.50 (dd, 1 H, *J* = 2.9 Hz, *J* = 10.0 Hz). − MS (CI; NH_4^+); *m/z* (%): 636 (100) [M + 18], 604 (5) [M − Me]. − $C_{29}H_{30}Br_2O_5$ (618.364): calcd. C 56.33, H 4.89; found C 56.54, H 4.80.

Methyl 2,3,4-Tri-*O*-benzyl-6,7-dideoxy-7-*C*-(2,3,4,6-tetra-*O*-benzyl-1-hydroxy-α,β-D-galactopyranosyl)-β-D-*galacto*-hept-6-ynopyranoside (6): A 2.5 M solution of butyllithium in hexane (7.76 mL, 19.4 mmol) was added to a solution of **3** (6.0 g, 9.7 mmol) in THF (100 mL) at −78°C under argon. The solution was stirred for 1.5 h at −10°C and then cooled to −78°C. A solution of **5** (5.2 g, 9.7 mmol) in THF (50 mL) was added at −78°C. The mixture was allowed to reach room temp., and stirred for 1.5 h, poured in iced water (200 mL), and extracted with dichloromethane (3 × 80 mL). The organic layer was dried ($MgSO_4$) and concentrated. The residue was chromatographed (cyclohexane/AcOEt, 6:1) to give **6** (5.2 g, 75% α.β = 1:1). − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40−7.10 (m, 35 H), 4.90−4.35 (m, 14 H), 4.23 (d, 0.5 H, *J* = 7.5 Hz), 4.21 (d, 0.5 H, *J* = 7.6 Hz), 3.88 (dd, 0.5 H, *J* = 7.6 Hz, *J* = 10.0 Hz), 3.85 (dd, 0.5 H, *J* = 7.6 Hz, *J* = 9.7 Hz), 3.46 (dd, 0.5 H, *J* = 10.0 Hz, *J* = 2.7 Hz), 3.38 (dd, 0.5 H, *J* = 9.7 Hz, *J* = 3.0 Hz), 4.10−3.60 (m, 4 H), 3.52 (s, 1.5 H), 3.60 (s, 1.5 H), 1.70 (s, 1 H). − ^{13}C NMR (100.60 MHz, $CDCl_3$): δ = 138.63−137.74 (C-arom), 128.68−127.31, 104.68, 104.62, 95.89, 92.05, 84.77, 82.56, 81.43, 79.24, 81.20−65.28, 75.68−72.56, 57.13, 56.90. − MS (CI; NH_4^+); *m/z* (%): 1014 (100) [M + 18], 996 (3) [M], 980 (25) [M − OH], 906 (50) [M − Bn]. − $C_{63}H_{64}O_{11}$ × 0.5 H_2O (1006.200): calcd. C 75.20, H 6.51; found C 75.29, H 6.33.

Methyl 2,3,4-Tri-*O*-benzyl-6,7-dideoxy-7-*C*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-β-D-*galacto*-hept-6-ynopyranoside (7): BF_3 × Et_2O (2.1 mL, 22.8 mmol) was added to a mixture of **6** (5.9 g, 5.9 mmol), triethylsilane (5 mL), anhydrous dichloromethane (20 mL), and anhydrous acetonitrile (150 mL). The reaction mixture was concentrated, and the residue chromatographed (cyclohexane/AcOEt, 8:1) to give **7** (5.7 g, 82%), m.p. 92−93°C. (cyclohexane/AcOEt).− $[\alpha]_D$ = +7.5 (*c* = 1.0, chloroform). − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.50−7.20 (m, 35 H), 5.15−4.60 (m, 14 H), 4.34 (d, 1 H, *J* = 7.6 Hz), 4.15 (d, 1 H, *J* = 1.0 Hz), 4.09 (dd, 1 H, *J* = 1.5 Hz, *J* = 9.5 Hz), 4.04 (d, 1 H, *J* = 9.0 Hz), 4.01 (d, 1 H, *J* = 3.0 Hz, *J* < 0.5 Hz), 3.78 (dd, 1 H, *J* = 7.6 Hz, *J* = 9.6 Hz), 3.75 (dd, 1 H, *J* = 1.0 Hz, *J* = 3.0 Hz), 3.60 (dd, 1 H, *J* = 9.5 Hz, *J* = 4.5 Hz), 3.58 (dd, 1 H, *J* = 1.5 Hz, *J* = 4.5 Hz, *J* < 0.5 Hz), 3.57 (dd, 1 H, *J* = 9.0 Hz, *J* = 9.0 Hz), 3.55 (s, 3 H, H-Me), 3.52 (dd, 1 H, *J* = 3.0 Hz, *J* = 9.0 Hz), 3.38 (dd, 1 H, *J* = 9.6 Hz, *J* = 3.0 Hz). − ^{13}C NMR (62.89 MHz, $CDCl_3$): δ = 138.75−137.71, 128.52−127.30, 104.71, 83.35−70.19, 81.36, 74.91, 65.61, 56.90. − MS (CI; NH_4^+); *m/z* (%): 998 (100) [M + 18], 968 (3) [M − OMe]. − $C_{63}H_{64}O_{10}$ × 0.5 H_2O (989.257): calcd. C 76.42, H 6.62; found C 76.54, H 6.71.

Methyl 6-*C*-(2,6-Anhydro-1-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl)-6-deoxy-β-D-galactopyranoside (8): A mixture of **7** (500 mg, 0.5 mmol) and 10% Pd/C (30 mg) in a mixture of ethyl acetate (4 mL) and methanol (10 mL) was stirred under hydrogen until deprotection of **7** was complete. The suspension was filtered through Celite and concentrated. The residue was crystallized in methanol to afford **8** (162 mg, 90%), m.p. 203°C (methanol). − $[\alpha]_D$ = −10 (*c* = 1, methanol). − ^{1}H NMR (400 MHz, D_2O): δ = 4.28 (d, 1 H, *J* = 7.9 Hz), 3.93 (dd, 1 H, *J* = 3.5, *J* < 0.5 Hz), 3.82 (dd, 1 H, *J* = 3.5, *J* < 0.5 Hz), 3.71 (m, 2 H), 3.62 (dd, 1 H, *J* = 3.5, *J* = 10.0 Hz), 3.61 (m, 1 H), 3.59 (dd, 1 H, *J* = 9.5, *J* = 3.5 Hz), 3.45 (dd, 1 H, *J* = 7.9, *J* = 10.0 Hz), 3.42 (t, 1 H, *J* = 9.5, *J* = 9.5 Hz), 3.35 (s, 1 H), 3.25 (dt, 1 H, *J* = 9.5, *J* = 9.5 Hz, *J* = 2.5 Hz), 2.05−1.50 (m, 4 H). − MS (CI; NH_4^+); *m/z* (%): 372 (100) [M + 18], 355 (35) [M + 1], 340 (20) [− Me], 323 (35) [− OMe]. − $C_{14}H_{26}O_{10}$ (354.353): calcd. C 47.45, H 7.40; found C 47.42, H 7.62.

Methyl 6-*C*-(2,6-Anhydro-3,4,5,7-tetra-*O*-benzyl-1-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl)-2,3,4-tri-*O*-benzyl-6-deoxy-β-D-galactopyranoside (9): A suspension of **7** (4.5 g, 4.5 mmol) and platinum(IV) oxide in ethyl acetate (100 mL) was stirred under hydrogen (1 atm). After complete disappearance of **7**, ethyl acetate (100 mL) was added; the suspension was filtered through Celite and concentrated. Chromatography of the residue (cyclohexane/AcOEt, 5:1) afforded **9** (4.5 g, 93%), m.p. 104.5−105°C (cyclohexane/AcOEt). − $[\alpha]_D$ = −4 (*c* = 1.0, chloroform). − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44−7.15 (m, 35 H), 5.00−4.50 (m, 14 H), 4.24 (d, 1 H, *J* = 7.6 Hz), 4.08 (d, 1 H, *J* < 0.5 Hz, *J* = 1.1 Hz, *J* < 0.5 Hz, H-6′), 3.83 (dd, 1 H, *J* = 7.6 Hz, *J* = 9.7 Hz), 3.65 (m, 1 H), 3.62 (dd,1 H, *J* = 9.8 Hz, *J* = 2.9 Hz), 3.61 (d, 1 H, *J* = 2.9 Hz, *J* < 0.5 Hz), 3.58 (m, 1 H), 3.54 (d, 1 H, *J* = 2.7 Hz, *J* < 0.5 Hz), 3.53 (m, 2 H), 3.52 (s, 3 H), 3.49 (dd, 1 H, *J* = 9.7 Hz, *J* = 2.7 Hz), 3.27 (m, 2 H, *J* = 8.2 Hz, *J* = 9.8 Hz), 2.10−1.40 (m, 4 H). − MS (CI; NH_4^+); *m/z* (%): 1002 (100) [M + 18], 970 (3) [M − OMe], 912 (3) [M − Bn]. − $C_{63}H_{68}O_{10}$ (985.224): calcd. C 76.80, H 6.96; found C 76.67, H 6.78.

6-*C*-(2,6-Anhydro-3,4,5,7-tetra-*O*-benzyl-1-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl)-2,3,4-tri-*O*-benzyl-6-deoxy-α,β-D-

galactopyranose (10): 3 M aq. sulfuric acid (2 mL) was added to a solution of **9** (3.5 g, 3.55 mmol) in acetic acid (100 mL) at 80°C. The mixture was stirred at this temperature until complete disappearance of **5**. Iced water (200 mL) was added. The reaction mixture was extrated with CH_2Cl_2 (3 × 80 mL). The organic layer was washed with sat. aq. $NaHCO_3$ (3 × 50 mL), dried ($MgSO_4$), and concentrated. The residue was chromatographed (cyclohexane/AcOEt, 5:1) to give **10** (2.45 g, 71%). − MS (CI; NH_3); *m/z* (%): 988 (100) [M + 18], 971 (15) [M + 1], 940 (75) [M − OMe]. − $C_{62}H_{66}O_{10}$ (971.197): calcd. C 76.68, H 6.85; found C 76.58, H 6.70.

6-*C*-(2,6-Anhydro-3,4,5,7-tetra-*O*-benzyl-1-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl)-2,3,4-tri-*O*-benzyl-6-deoxy-D-galactono-1,5-lactone (11): PCC (0.66 g, 3 mmol) was added to a solution of **10** (2.45 g, 2.5 mmol) in dichloromethane in the presence of 4-Å molecular sieves. The suspension was stirred for 2 h, filtered through Celite and concentrated. Column chromatography of the residue afforded **11** (2.1 g, 87%). − $[\alpha]_D$ = +45 (*c* = 1.0, chloroform). − ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.50−7.10 (m, 35 H), 5.25 (d, 1 H, *J* = 11.0 Hz), 5.00−4.60 (m, 14 H), 4.50−4.40 (m, 2 H), 4.30 (dd, 1 H, *J* = 7.0 Hz, *J* = 7.0 Hz, *J* < 0.5 Hz), 4.00 (d, 1 H, *J* = 2.9 Hz, *J* < 0.5 Hz), 3.85 (m, 2 H), 3.68 (dd, 1 H *J* = 8.5 Hz, *J* = 7.9 Hz), 3.62 (dd, 1 H, *J* = 2.8 Hz, *J* = 11.0 Hz), 3.55 (dd, 1 H, *J* = 2.9 Hz, *J* = 8.5 Hz), 3.20 (dd, 1 H, *J* = 7.9 Hz, *J* = 7.9 Hz, *J* < 0.5 Hz), 2.00−1.30 (m, 4 H). − ^{13}C NMR (62.89 MHz, $CDCl_3$): δ = 170.38, 138.66−137.59, 128.35−127.38, 84.65, 80.67−72.81, 68.72, 27.35, 27.38. − MS (CI; NH_3); *m/z* (%): 986 (100) [M + 18], 969 (5) [M + 1], 936 (20) [M − OMe], 877 (80) [M − Bn]. − $C_{62}H_{64}O_{10}$ (969.182): calcd. C 76.84, H 6.66; found C 77.16, H 6.51.

Methyl 7-*C*-[6-*C*-(2,6-Anhydro-3,4,5,7-tetra-*O*-benzyl-1-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl)-2,3,4-tri-*O*-benzyl-6-deoxy-1-hydroxy-α,β-D-galactopyranosyl]-2,3,4-tri-*O*-benzyl-6,7-dideoxy-β-D-*galacto*-hept-6-ynopyranoside (12): 11 (1.35 g, 2.2 mmol) was treated with the lithio derivative **4** as described for **6** to give **12** (2.1 g, 67%), which was directly engaged in the next step.

Methyl 7-*C*-[6-*C*-(2,6-Anhydro-3,4,5,7-tetra-*O*-benzyl-1-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl)-2,3,4-tri-*O*-benzyl-6-deoxy-β-D-galactopyranosyl]-2,3,4-tri-*O*-benzyl-6,7-dideoxy-β-D-*galacto*-hept-6-ynopyranoside (13): 12 (2.1 g, 1.46 mmol) was treated with triethylsilane (1.4 mL, 8.8 mmol) as described for **7** to give **13** (1.77 g, 86%). − $[\alpha]_D$ = +10 (*c* = 1.0, chloroform). − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.50−7.15 (m, 50 H), 5.05−4.50 (m, 20 H), 4.27 (d, 1 H, *J* = 7.8 Hz), 4.14 (s, 1 H, *J* < 0.5 Hz), 4.06 (dd, 1 H, *J* = 9.6 Hz, *J* = 7.9 Hz), 4.05 (d, 1 H, *J* = 7.9 Hz), 4.03 (s, 1 H, *J* < 0.5 Hz, *J* < 0.5 Hz, *J* < 0.5 Hz), 3.85 (dd, 1 H, *J* = 9.8 Hz, *J* = 7.8 Hz), 3.84 (d, 1 H, *J* = 3.0 Hz, *J* < 0.5 Hz), 3.70 (d, 1 H, *J* = 2.5 Hz, *J* < 0.5 Hz), 3.65 (dd, 1 H, *J* = 9.8 Hz, *J* = 7.9 Hz), 3.64 (d, 1 H, *J* = 3.0 Hz, *J* < 0.5 Hz), 3.61 (dd, 1 H, *J* = 9.8 Hz, *J* = 3.0 Hz), 3.60−3.58 (m, 2 H), 3.56 (s, 3-H), 3.49 (dd, 1 H, *J* = 9.6 Hz, *J* = 2.5 Hz), 3.42 (dd, 1 H, *J* = 9.8 Hz, *J* = 3.0 Hz), 3.28 (t, 1 H, *J* < 0.5 Hz, *J* = 6.5 Hz, *J* = 6.5 Hz), 3.19 (ddd, 1 H, *J* = 7.9 Hz, *J* = 7.9 Hz, *J* = 1.0 Hz), 2.0−1.4 (m, 4 H). − ^{13}C NMR (62.89 MHz, $CDCl_3$): δ = 130.66−137.98, 128.69−127.44, 104.81, 84.66−68.86, 75.53−73.54, 65.77, 57.02, 28.03, 27.41. − MS (CI; NH_3); *m/z* (%): 1428 (100) [M + 18], 1337 (5) [− Bn], 1320 (15) [M − Bn], 1287 (15) [−MeOH]. − $C_{91}H_{94}O_{14}$ (1411.745): calcd. C 77.42, H 6.71; found C 77.38, H 6.82.

Methyl 6-*C*-[2,6-Anhydro-6-*C*-(2,6-anhydro-1-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl)-1-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl]-6-deoxy-β-D-galactopyranoside (14): 13 (500 mg, 0.35 mmol) was treated with hydrogen as described for **8** to give **14** (160 mg, 89%), m.p. 187°C (dec.). − $[\alpha]_D$ = −4 (*c* = 0.2, methanol). − ^{1}H NMR (400 MHz, D_2O): δ = 4.28 (d, 1 H, *J* = 7.9 Hz), 3.94 (d, 1 H, *J* = 3.5 Hz, *J* < 0.5 Hz), 3.86 (d, 1 H, *J* = 3.5, *J* < 0.5, H-5″), 3.82 (d, 1 H, *J* = 3.5, *J* < 0.5), 3.72 (m, 2 H), 3.60 (m, 5 H), 3.61 (dd, 1 H, *J* = 9.8 Hz, *J* = 3.5 Hz), 3.52 (s, 3 H), 3.46 (dd, 1 H, *J* = 7.9 Hz, *J* = 9.8 Hz), 3.40 (2 t, 2 H, *J* = 9.5 Hz, *J* = 9.5 Hz, *J* = 9.5 Hz, *J* = 9.5 Hz), 3.25 (m, 2 H, *J* = 9.5 Hz, *J* = 9.5 Hz, *J* = 2.5 Hz, *J* = 9.5 Hz, *J* = 9.5 Hz, *J* = 2.5 Hz), 2.05−1.55 (m, 8 H). − MS (CI; NH_3); *m/z* (%): 532 (25) [M + 18], 515 (5) [M + 1], 500 (10) [M − Me], 483 (100) [M − OMe], 465 (20) [− H_2O], 447 (20) [− 2 H_2O], 429 (15) [− 3 H_2O], 411 (15) [− 4 H_2O]. − $C_{21}H_{38}O_{14}$ × 1.5 H_2O (541.550): calcd. C 46.58, H 7.63; found C 46.52, H 7.56.

Methyl 6-*C*-[2,6-Anhydro-6-*C*-(2,6-anhydro-3,4,5,7-tetra-*O*-benzyl-1-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl)-3,4,5-tri-*O*-benzyl-1-deoxy-β-D-*glycero*-L-*manno*-hepitol-1-yl]-2,3,4-tri-*O*-benzyl-6-deoxy-β-D-galactopyranoside (15): 13 (1.1 g, 0.78 mmol) was hydrogenated as described for **7** to give **15** (1.08 g, 96%). − $[\alpha]_D$ = −1.5 (*c* = 1.0, chloroform). − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.50−7.15 (m, 50 H), 5.05−4.50 (m, 20 H), 4.27 (d, 1 H, *J* = 7.8 Hz), 4.14 (s, 1 H, *J* < 0.5 Hz), 4.06 (dd, 1 H, *J* = 9.6 Hz, *J* = 7.9 Hz), 3.85 (2 dd, 2 H, *J* = 7.8 Hz), 3.44 (dd, 1 H, *J* = 3.0, *J* = 9.5 Hz), 3.28 (ddd, 1 H, *J* = 8.5 *J* = 8.5, *J* < 1 Hz), 3.18 (ddd, 1 H, *J* = 8.8, *J* = 8.8, *J* < 1 Hz), 1.92−1.35 (m, 8 H). − MS (CI; NH_3); *m/z* (%): 1432 (100) [M + 18]. − $C_{91}H_{98}O_{14}$ (1415.767): calcd. C 77.20, H 6.98; found C 77.40, H 7.00.

6-*C*-[2,6-Anhydro-6-*C*-(2,6-anhydro-3,4,5,7-tetra-*O*-benzyl-1-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl-)-3,4,5-tri-*O*-benzyl-1-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl]-2,3,4-tri-*O*-benzyl-6-deoxy-α,β-D-galactopyranose (16): 15 (1.0 g, 0.71 mmol) was treated with sulfuric acid in acetic acid as described for **9** to give **16** (505 mg, 51%). − $C_{90}H_{96}O_{14}$ (1401.74): calcd. C 77.12, H 6.90; found C 77.05, H 7.20.

6-*C*-[2,6-Anhydro-6-*C*-(2,6-anhydro-3,4,5,7-tetra-*O*-benzyl-1-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl)-3,4,5-tri-*O*-benzyl-1-deoxy-β-D-*glycero*-L-*manno*-hepitol-1-yl]-2,3,4-tri-*O*-benzyl-6-deoxy-D-galactopyranolactone (17): 16 (500 mg, 0.36 mmol) was oxidized as described for **10** to give **17** (450 mg, 88%). − $[\alpha]_D$ = +33.5 (*c* = 1, chloroform). − ^{13}C NMR (62.89, $CDCl_3$): δ = 170.59, 138.86−137.96, 126.43−127.25, 84.95−72.11. − $C_{90}H_{94}O_{14}$ (1399.724): calcd. C 77.23, H 6.77; found C 77.09, H 6.58.

Methyl 7-*C*-{6-*C*-[2,6-Anhydro-6-*C*-(2,6-anhydro-3,4,5,7-tetra-*O*-benzyl-1-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl)-3,4,5-tri-*O*-benzyl-1-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl]-2,3,4-tri-*O*-benzyl-6-deoxy-1-hydroxy-α,β-D-galactopyranosyl}-2,3,4-tri-*O*-benzyl-6,7-dideoxy-β-D-*galacto*-hept-6-ynopyranoside (18): 17 (400 mg, 0.32 mmol) was condensed with the lithio derivative **4** (200 mg, 0.32 mmol) as described for **5** to give **18** (406 mg, 68%), which was directly engaged in the next step.

Methyl 7-*C*-{6-*C*-[2,6-Anhydro-6-*C*-(2,6-anhydro-3,4,5,7-tetra-*O*-benzyl-1-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl)-3,4,5-tri-*O*-benzyl-6-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl]-2,3,4-tri-*O*-benzyl-6-deoxy-β-D-galactopyranosyl}-2,3,4-tri-*O*-benzyl-6,7-dideoxy-β-D-*galacto*-hept-6-ynopyranoside (19): 18 (400 mg, 0.22 mmol) was reduced as described for **6** to give **19** (309 mg, 78%). − $[\alpha]_D$ = +8 (*c* = 1.0, $CHCl_3$). − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.52−7.15 (m, 65 H), 5.00−4.48 (m, 26 H), 4.23 (d, 1 H, *J* = 7.9 Hz), 4.08 (s, 1 H, *J* < 0.5 Hz), 4.07 (d, 1 H, *J* = 7.8 Hz), 4.00 (dd, 1 H, *J* = 7.8 Hz, *J* = 9.8 Hz), 3.98 (s, 1 H, *J* < 0.5 Hz, *J* < 0.5 Hz, *J* < 0.5 Hz), 3.81 (dd, 1 H, *J* = 7.8 Hz, *J* = 9.8 Hz), 3.78 (d, 1 H, *J* = 3.0 Hz, *J* < 0.5 Hz), 3.68 (d, 1 H, *J* = 2.5 Hz, *J* < 0.5 Hz), 3.65 (dd, 2 H, *J* = 9.8 Hz, *J* = 7.8 Hz, *J* = 9.8 Hz, *J* = 7.8 Hz), 3.65−3.52 (m, 6 H), 3.52 (s, 3 H), 3.44 (dd, 1 H, *J* = 9.8 Hz, *J* = 2.8 Hz), 3.40

(dd, 1 H, J = 9.8 Hz, J = 3.0 Hz), 3.23 (dd, 1 H, J = 6.0 Hz, J = 7.6 Hz, J < 0.5 Hz), 3.18 (dd, 1 H, J = 7.8 Hz, J = 7.8 Hz, J < 0.5 Hz), 3.15 (dd, 1 H, J < 0.5 Hz, J = 6.5 Hz, J = 6.5 Hz), 3.10 (ddd, 1 H, J = 7.8 Hz, J = 7.8 Hz, J = 1.5 Hz), 2.00−1.40 (m, 8 H). − MS (CI; NH_3); *m/z* (%): 1858 (100) [M + 18], 1767 (25) [− Bn], 1752 (35) [− Me], 1661 (45) [− Bn]. − $C_{119}H_{124}O_{18} \times 0.5\ H_2O$ (1851.286): calcd. C 77.20, H 6.81; found C 77.04, 6.93.

Methyl 6-*C*-{2,6-Anhydro-6-*C*-[2,6-anhydro-6-*C*-(2,6-anhydro-β-D-*glycero*-L-*manno*-heptitol-1-yl)-6-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl]-6-deoxy-β-D-*glycero*-L-*manno*-heptitol-1-yl}-6-deoxy-β-D-galac-topyranoside (20): **19** (300 mg, 0.17 mmol) was treated with hydrogen as described for **8** to give **20** (100 mg, 91%), m.p. 154°C (dec.). − $[\alpha]_D$ = −4 (c = 1, methanol/water, 3:2 v/v). − ^{1}H NMR (400 MHz, D_2O): δ = 4.28 (d, 1 H, J = 7.9 Hz), 3.94 (d, 1 H, J < 0.5 Hz, J = 3.5 Hz), 3.86 (d, 2 H, J = 3.5 Hz, J < 0.5 Hz, J < 0.5 Hz, J = 3.5 Hz), 3.82 (d, 1 H, J < 0.5 Hz, J = 3.5 Hz), 3.74 (dd, 1 H, J = 7.9 Hz, J = 11.5 Hz), 3.68 (dd, 1 H, J = 4.3 Hz, J = 11.5 Hz), 3.63 (dd, 1 H, J = 3.5 Hz, J = 9.8 Hz), 3.58 (dd, 3 H, J = 3.5 Hz, J = 9.8 Hz, J = 3.5 Hz, J = 9.8 Hz, J = 3.5 Hz, J = 9.8 Hz), 3.56 (m, 3 H), 3.52 (dd, 1 H, J < 0.5 Hz, J = 7.9 Hz, J = 4.3 Hz), 3.47 (dd, 1 H, J = 7.9 Hz, J = 9.8 Hz), 3.39 (t, 2 H, J = 9.8 Hz, J = 9.8 Hz, J = 9.8 Hz, J = 9.8 Hz), 3.38 (t, 1 H, J = 9.8 Hz, J = 9.8 Hz), 3.34 (s, 3 H), 3.27 (dt, 1 H, J = 9.8, J = 9.8, J = 1.0 Hz), 3.25 (dt, 1 H, J = 9.8 Hz, J = 9.8 Hz, J = 1.0 Hz), 3.22 (dt, 1 H, J = 9.8 Hz, J = 9.8 Hz, J = 1.0 Hz), 2.10−1.48 (3 m, 12 H). − MS (CI; NH_3); *m/z* (%): 692 (100) [M + 18], 674 (5) [M], 660 (40) [M − Me], 643 (20) [M − MeOH]. − $C_{28}H_{50}O_{18} \times 2.5\ H_2O$ (719.731): calcd. C 46.73, H 7.70; found C 46.82, H 7.85.

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