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Diversity-oriented Synthesis of Inner Core Oligosaccharides of the Lipopolysaccharide of Pathogenic Gram-negative Bacteria

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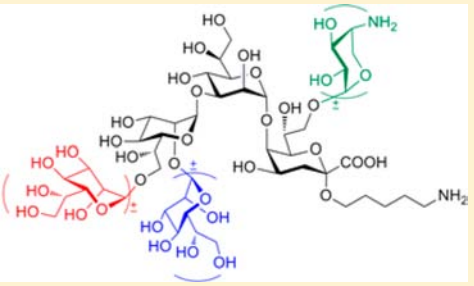
Supporting Information

ABSTRACT: Lipopolysaccharide (LPS) is a potent virulence factor of pathogenic Gram-negative bacteria. To better understand the role of LPS in host−pathogen interactions and to elucidate the antigenic and immunogenic properties of LPS inner core region, a collection of well-defined L-*glycero*-D-*manno*-heptose (Hep) and 3-deoxy-α-D-*manno*-oct-2-ulosonic acid (Kdo)-containing inner core oligosaccharides is required. To address this need, we developed a diversity-oriented approach based on a common orthogonal protected disaccharide Hep-Kdo. Utilizing this new approach, we synthesized a range of LPS inner core oligosaccharides from a variety of pathogenic bacteria including *Y. pestis*, *H. influenzae*, and *Proteus* that cause plague, meningitis, and severe wound infections, respectively. Rapid access to these highly branched core oligosaccharides relied on elaboration of the disaccharide Hep-Kdo core as basis for the elongation with various flexible modules including unique Hep and 4-amino-4-deoxy-β-L-arabinose (Ara4N) monosaccharides and branched Hep-Hep disaccharides. A regio- and stereoselective glycosylation of Kdo 7,8-diol was key to selective installation of the Ara4N moiety at the 8-hydroxyl group of Kdo moiety of the Hep-Kdo disaccharide. The structure of the LPS inner core oligosaccharides was confirmed by comparison of ^{1}H NMR spectra of synthetic antigens and isolated fragments. These synthetic LPS core oligosaccharides can be covalently bound to carrier proteins via the reducing end pentyl amine linker, to explore their antigenic and immunogenic properties as well as potential applications such as diagnostic tools and vaccines.



INTRODUCTION

Many Gram-negative bacteria are notorious pathogens that can lead to a variety of infectious diseases, such as meningitis, pneumonia and plague.[1] The pathogenicity and virulence of Gram-negative bacteria are often associated with the lipopolysaccharide (LPS) coat.[2,3] LPS, a highly complex glycolipid, is the major component the outer membrane of Gram-negative bacteria.[3] Elucidation of the role of LPS is essential for understanding the host−pathogen interactions during the infection of Gram-negative bacteria. As a potent virulence factor, LPS also serves as a surface pathogen-associated antigen for recognition by the host immune system.[3] Therefore, LPS has attracted much interest for the development of diagnostic tools, therapeutic reagents and vaccine candidates.

LPS is comprised of the lipid A moiety, the core oligosaccharide, and the *O*-specific polysaccharide. The core oligosaccharide, connecting lipid A with the outer *O*-antigen, is present in every natural LPS structure. The *O*-antigen in turn may be missing in many pathogenic bacteria such as *N. meningitidis*, *H. influenzae* and *Y. pestis*.[4,5]

Structurally, the core region of LPS can be further subdivided into inner core and outer core. The main structural motifs of the inner core are highly conserved throughout the Gram-negative bacteria while the sugars of the outer core vary greatly between strains.[4] 3-Deoxy-α-D-*manno*-oct-2-ulosonic acid (Kdo) and L-*glycero*-D-*manno*-heptose (Hep) are ubiquitously present in most LPS structures (Figure 1). Another frequently detected sugar unit in the LPS is 4-amino-4-deoxy-β-L-arabinose (Ara4N).[6] Attachment of Ara4N to the LPS, via a glycosidic linkage at the *C*-8 position of Kdo, has been

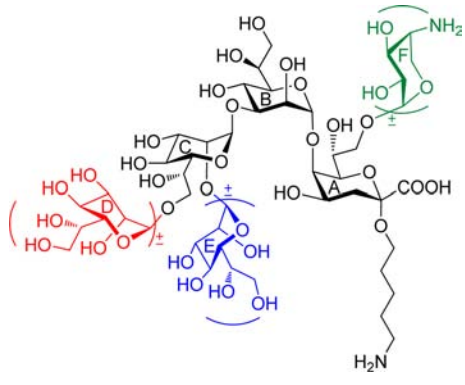


Figure 1. Conserved inner core oligosaccharides of LPS from *Y. pestis*, *H. influenzae* and *Proteus*. A, 3-Deoxy-α-D-*manno*-oct-2-ulosonic acid (Kdo); B−E, L-*glycero*-D-*manno*-heptose (Hep); F, 4-amino-4-deoxy-β-L-arabinose (Ara4N).

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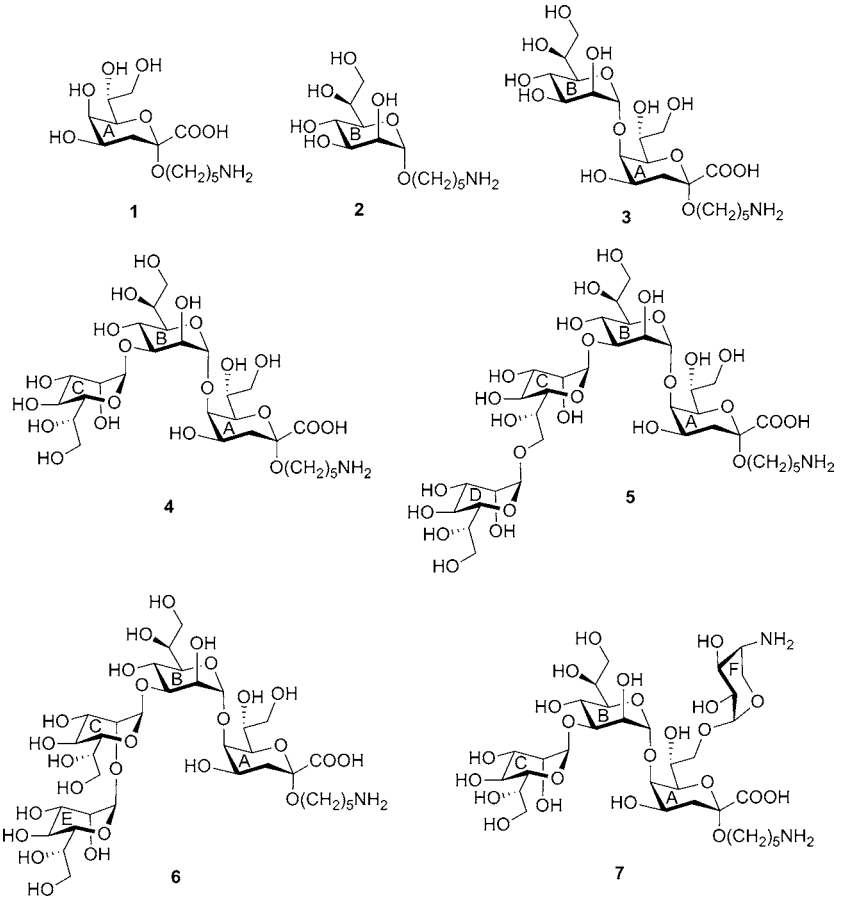

Figure 2. Synthetic LPS antigens for immunological studies.

implicated as a major factor of bacterial resistance against cationic antimicrobials, such as polymyxin B (PmB).[7]

Most Gram-negative bacteria share the trisaccharide L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo as a common conserved inner core structure (ABC in Figure 1).[8] This core can be decorated with other glycans, phosphates, or occasionally acetyl groups. Elongation of the core trisaccharide ABC at the *C*-7 position of the Hep moiety C with Hep residue D, gives rise to the conserved core tetrasaccharide L,D-Hep-(1→7)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo (ABCD) in *Y. pestis*.[9,10] Extension of the core trisaccharide ABC at the *C*-2 position of Hep C with Hep residue E, generates the conserved core tetrasaccharide ABCE L,D-Hep-(1→2)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo in *H. influenza*.[11] Affixing Ara4N residue F at the *C*-8 position of Kdo A within the core trisaccharide, furnishes the conserved core tetrasaccharide A(F)BC L,D-Hep-(1→3)-L,D-Hep-(1→5)-[L-Ara4N-(1→8)]-Kdo common to most *Proteus* strains.[6]

Synthetic approaches toward the LPS inner core region require the production of multigram quantities of the higher carbon sugars Hep and Kdo followed by transformation into suitable building blocks. Previous synthetic efforts toward LPS inner core oligosaccharides covered the basic structural motifs of the inner core region.[12,13] The LPS inner core structures are highly branched, and target-oriented synthesis is often not flexible enough to accommodate the synthesis of mutiple branched oligosaccharides. A general strategy for the diversity-oriented synthesis of a library of LPS inner core oligosaccharides would be ideal to rapidly access these molecules.

Here, we report the synthesis of LPS inner core oligosaccharides **1**-**7** using a general and flexible approach (Figure 2). Monosaccharides **1**, **2** as well as disaccharide **3** are basic structural elements of the LPS core region, and trisaccharide **4** is highly conserved in most Gram-negative bacteria. Tetrasaccharides **5**, **6**, and **7**, incorporating different branching points at the core trisaccharide **4**, are conserved in *Y. pestis*, *H. influenzae*, and *Proteus* bacteria. These well-defined synthetic LPS inner core oligosaccharides can be conjugated to carrier proteins for immunization studies. The resulting glycoconjugates help elucidate the antigenic and immunogenic properties as diagnostic markers and vaccine candidates. Furthermore, the structural basis for the interaction of LPS-specific antibodies directed against synthetic antigens (epitope mapping) can be enabled.[14] In addition, synthetic core antigens may serve as substrates for enzymes that add immunorelevant decorating groups onto the core region.[7,15]

■ RESULTS AND DISCUSSION

The chemical synthesis of several LPS oligosaccharides is challenging as it requires the formation of various glycosidic linkages within the steric constraints of the highly branched inner core region. To date, target-oriented strategies have been employed for the synthesis of structurally closely related molecules.[12,13] The efficient synthesis of diverse LPS inner core oligosaccharides necessitates a general, diversity-oriented strategy relying on common modules.[16]

It was envisaged for oligosaccharides **4**−**7** to be prepared from common disaccharide **8** that is decorated with the orthogonal protecting groups levulinate (Lev)[17] and isopropylidene acetal,[18] respectively (Scheme 1). Disaccharide **8** can be

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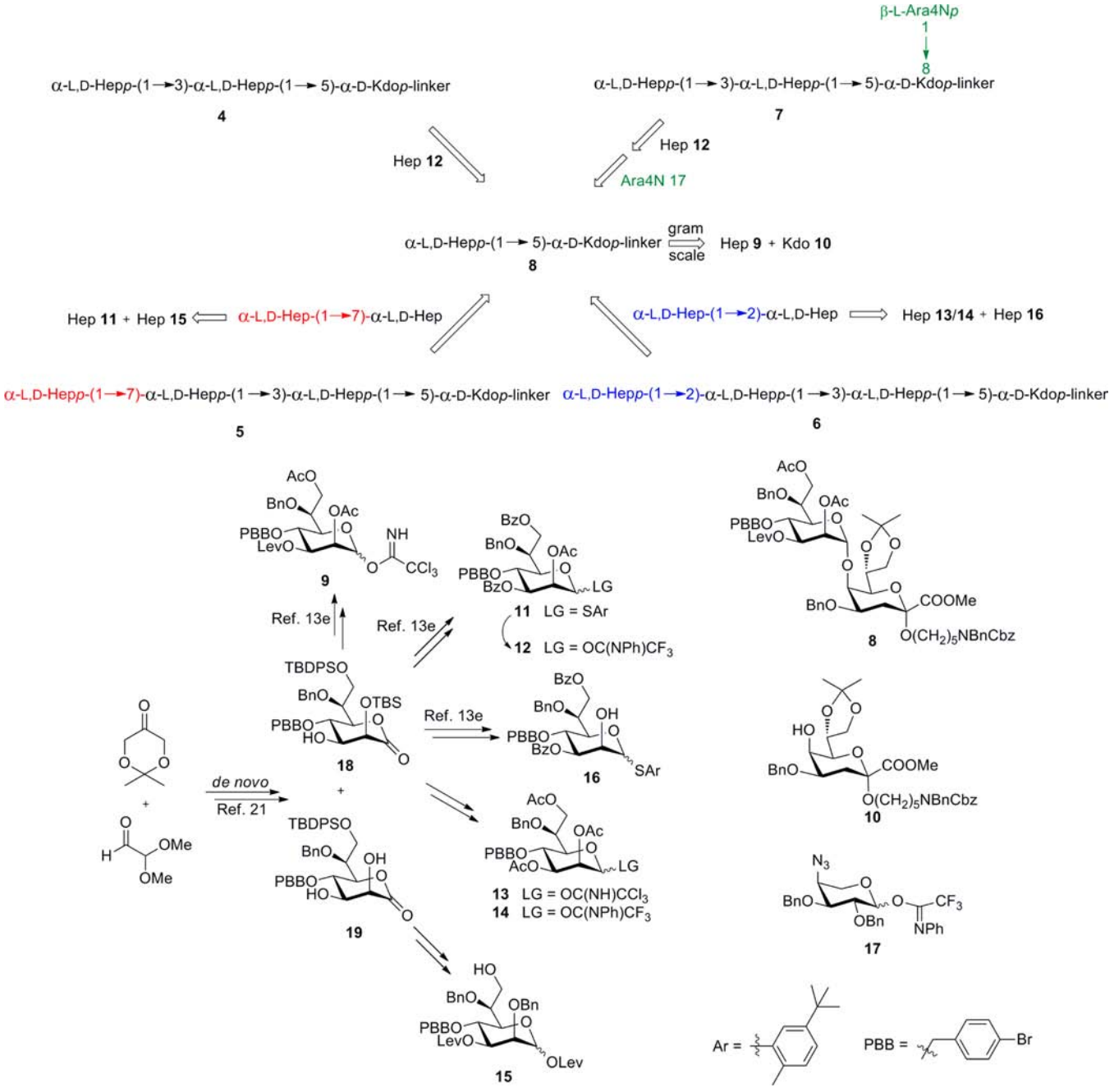
Scheme 1. Retrosynthetic Analysis of LPS Core Oligosaccharides 4−7



synthesized in gram scale by coupling heptose trichloroacetimidate[19] **9** with Kdo building block **10**. Thus, a foundation for the extension with various monosaccharide and branched disaccharide residues will be laid. The Kdo moiety of disaccharide **8** possesses a *N*-benzyl-*N*-benzyloxycarbonyl pentyl linker[20] for further conjugation to a carrier protein.

Flexible building blocks to install highly branched sugar residues of LPS include Hep building blocks **11**−**16**, and Ara4N building block **17**[13d] (Scheme 1). All required Hep building blocks **11**−**16** were to be accessed by modification of Hep intermediates **18** and **19** obtained by a *de novo* synthesis we developed recently.[13e,21] Based on common disaccharide **8**, installation of the terminal α-linked Hep residue of **4** should be straightforward by using building blocks **11** or **12**. Removal of all protecting groups of the newly formed trisaccharide is expected to give target trisaccharide **4**. The α-(1→7) linked diheptose moiety of **5** will be constructed by employing Hep building blocks **11** and **15**. The α-(1→2) linked diheptose moiety of **6** will be prepared by glycosylation of Hep building blocks **13** or **14** with Hep **16**. The resulting α-(1→7) and α-(1→2) linked diheptoses are converted to the glycosyl *N*-phenyl trifluoroacetimidate[22] for [2 + 2] couplings with the building block produced by removal of the Lev group in **8**. The [2 + 2] couplings will rely on the anomeric effect[23] of Hep assisted by solvent effects[24] to ensure stereoselectivity and will give access to target tetrasaccharides **5** and **6** after global deprotection. The β-linked Ara4N moiety of **7** will be regio- and stereoselectively installed at C-8 position of the Kdo 7,8-vicinal diol derived from **8** and Ara4N building block **17**. The required β-(1→8) linked trisaccharide containing Ara4N will be

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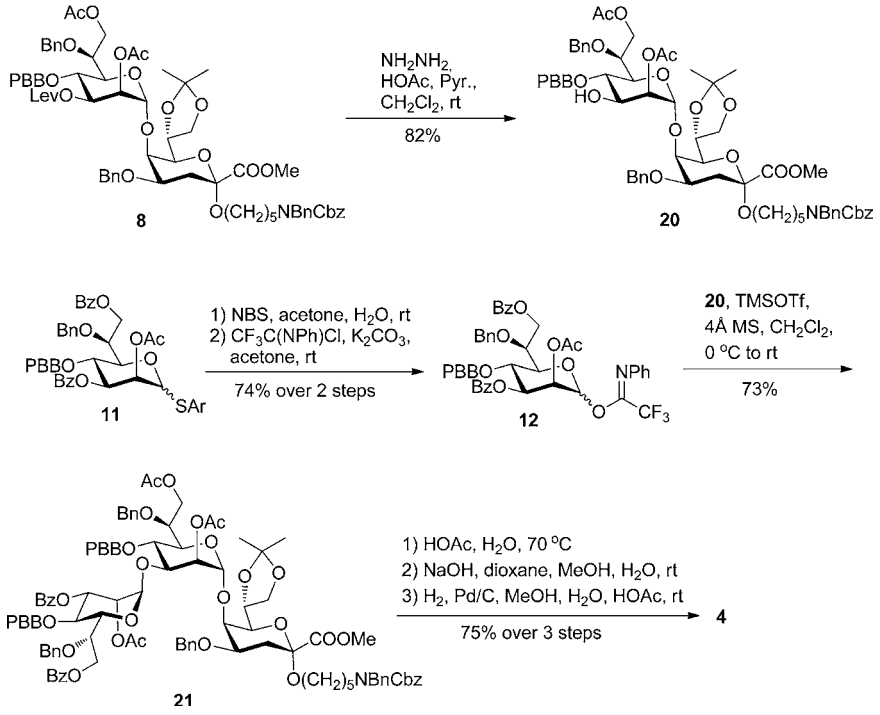
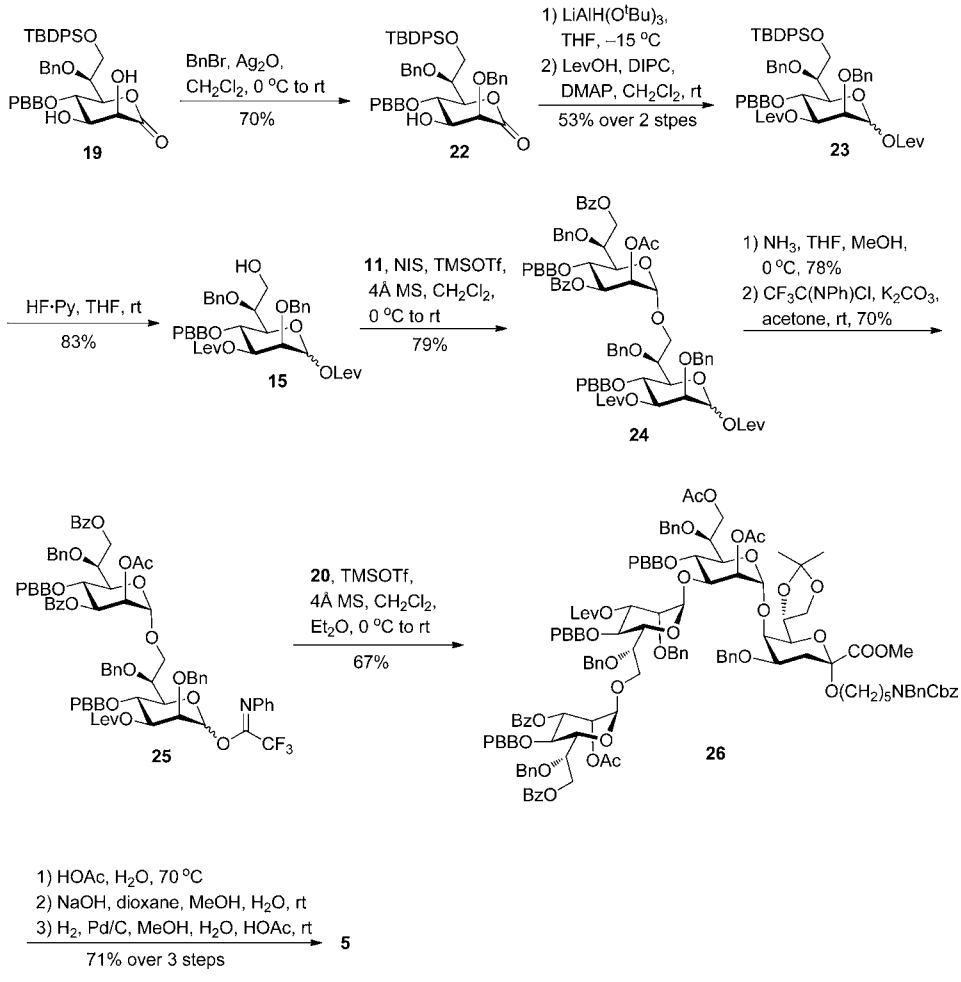
Scheme 2. Synthesis of the Conserved Inner Core Trisaccharide 4



Scheme 3. Synthesis of the Inner Core Tetrasaccharide 5 of *Y. pestis*



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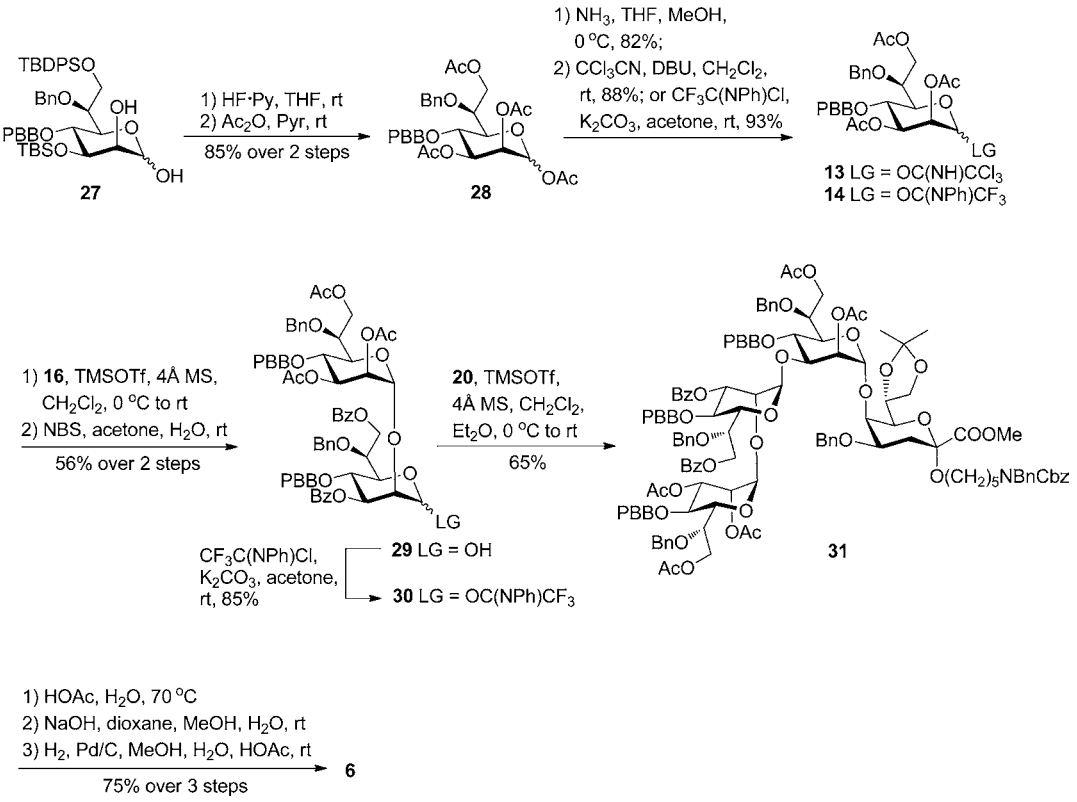
Scheme 4. Synthesis of the Inner Core Tetrasaccharide 6 of *H. influenzae*



transformed into a trisaccharide building block with a *C*-3 hydroxyl group after Lev cleavage. Heptosylation of the resulting trisaccharide with **12** will provide target tetrasaccharide **7** following global deprotection.

Executing on this plan, disaccharide **8** was prepared using Hep trichloroacetimidate **9** and Kdo building block **10**, employing an efficient glycosylation protocol that was developed specifically to meet the challenge.[13e] For that purpose, protecting groups, anomeric leaving groups, and glycosylation conditions were fine-tuned for the construction of this α-(1→5) linked Hep-Kdo linkage. The robust glycosylation finally produced gram amounts of disaccharide **8**. Removal of the Lev group in **8** using hydrazine in a mixture of acetic acid, pyridine and dichloromethane gave disaccharide **20** in 82% yield (Scheme 2). The solvent mixture acts as a buffer such that all other ester groups remained and no side products were formed.

Hep thioglycoside **11**[13e] was transformed to the corresponding *N*-phenyl trifluoroacetimidate glycosylating agent **12** in 74% yield over two steps (Scheme 2). Condensation of Hep **12** with disaccharide **20** was catalyzed by TMSOTf and produced trisaccharide **21** in 73% yield as the α-anomer. The configuration of trisaccharide **21** was confirmed by the coupling constants between *C*-1 and *H*-1 of the corresponding heptoses ($^1J_{C-H}$ = 176.4 and 176.0 Hz, respectively).[25,26] Global deprotection of **21** was accomplished via a three-step procedure to afford trisaccharide **4**. Acidic cleavage of the isopropylidene acetal group with aqueous acetic acid at 70 °C was followed by saponification of all esters by reaction with aqueous sodium hydroxide. Finally, hydrogenolysis of the benzyl, *p*-bromobenzyl (PBB)[27] and benzyloxycarbonyl groups over Pd/C in a mixture of methanol and water with a catalytic amount of acetic acid produced **4** (75% over three steps).

The synthesis of tetrasaccharide **5** of *Y. pestis* was based on known intermediate **19**.[21] Selective benzylation of the *C*-2 position of **19** with benzyl bromide and silver(I) oxide gave lactone **22** in 70% yield (Scheme 3). Reduction of **22** with lithium tri-*tert*-butoxyaluminium hydride[28] followed by levulinoylation of both hydroxyl groups provided heptose **23** in 53% yield over two steps. Analysis of the NMR spectra of **23** confirmed the location of the benzyl and levulinoyl groups.[26] The silyl ether in **23** was cleaved using HF·pyridine to afford heptose **15**. Coupling of Hep **11** with Hep **15** using NIS and TMSOTf as promoters[29] proceeded smoothly to afford α-(1→7)-linked disaccharide **24** in 79% yield. Selective cleavage of the anomeric levulinate in **24** with ammonia,[30] followed by *N*-phenyl trifluoroacetimidate formation produced disaccharide building block **25**. A TMSOTf catalyzed [2 + 2] coupling of *N*-phenyl trifluoroacetimidate **25** and **20** furnished tetrasaccharide **26** in 67% yield. The desired configuration of tetrasaccharide **26** was confirmed by the coupling constants between *C*-1 and *H*-1 of the corresponding heptoses ($^1J_{C-H}$ = 174.0, 179.4 and 175.8 Hz, respectively).[26] Final deprotection of **26** involved acidic isopropylidene acetal cleavage, saponification, and hydrogenolysis to provide target tetrasaccharide **5** in 71% yield over three steps.

The synthesis of the α-(1→2)-linked diheptose moiety in tetrasaccharide **6** of *H. influenza* started from Hep **27**[21] that can be accessed by reduction of Hep intermediate **18** with lithium tri-*tert*-butoxyaluminium hydride. Cleavage of the silyl ethers in **27** with HF·pyridine and subsequent acetylation gave **28** in 85% yield (Scheme 4). Ester **28** was deacetylated selectively to afford the heptose hemiacetal that was treated with trichloroacetonitrile and *N*-phenyl trifluoroacetimidoyl chloride respectively to furnish Hep building blocks **13** and **14**. Coupling of Hep trichloroacetimidate **13** and Hep **16** produced

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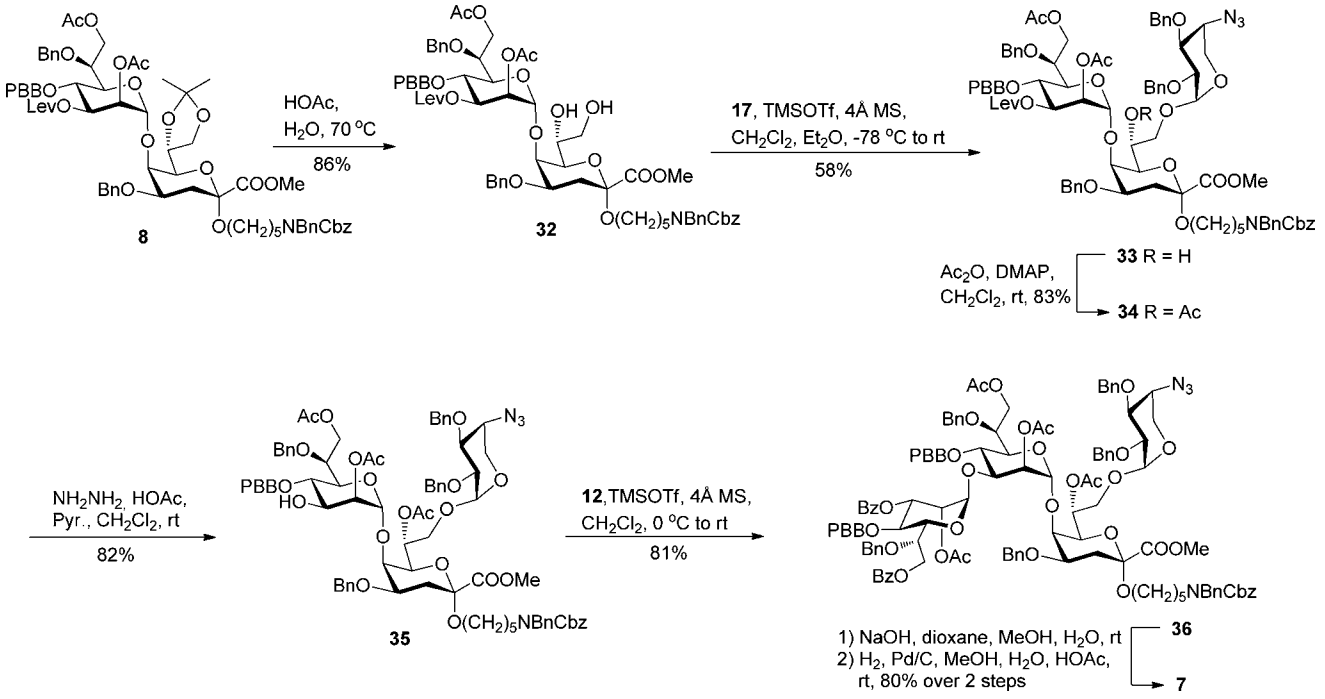
Scheme 5. Synthesis of the *Proteus* Inner Core Tetrasaccharide 7



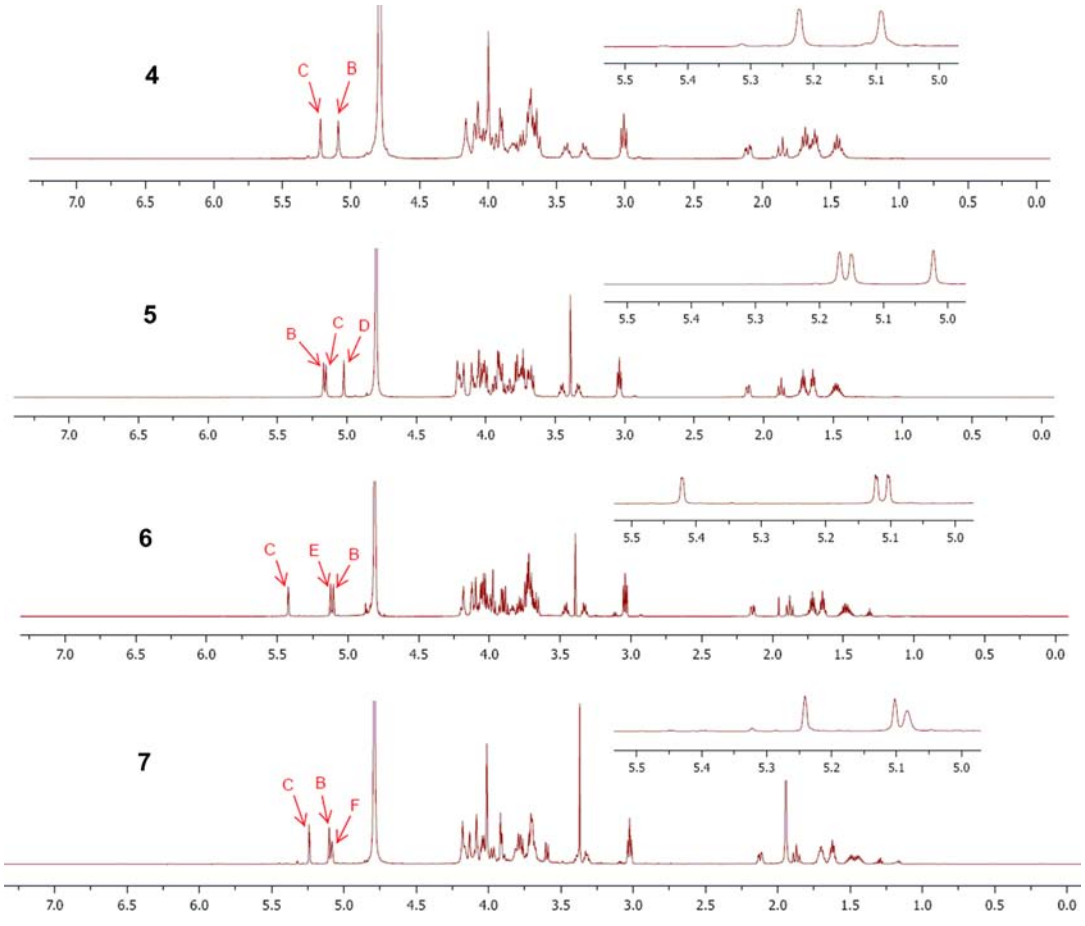


Figure 3. Confirmation of structural integrity of LPS core oligosaccharides **4**–**7** by NMR.

a disappointingly low yield of the α-(1→2)-linked disaccharide due to extensive hydrolysis of **13**. Coupling of Hep *N*-phenyl trifluoroacetimidate **14** and Hep **16** was more productive in the presence of TMSOTf. Treatment of the newly formed disaccharide with NBS gave disaccharide **29** in 56% yield over two steps, allowing for the separation of disaccharide **29**

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from the remaining derivatives of monosaccharide **16** at this step. Disaccharide hemiacetal **29** was converted into *N*-phenyl trifluoroacetimidate **30** that engaged in a [2 + 2] coupling with disaccharide **20**. TMSOTf activation of **30** in CH_2Cl_2/Et_2O provided the desired α-linked tetrasaccharide **31** in 65% yield. The three step global deprotection of **31** gave target tetrasaccharide **6** in 75% yield over three steps. The configuration of tetrasaccharide **6** was confirmed by the coupling constants between *C*-1 and *H*-1 of the corresponding heptoses ($^1J_{C-H}$ = 173.4, 172.2 and 175.8 Hz, respectively).[26]

Synthetic work toward the tetrasaccharide **7** of *Proteus* commenced with the removal of the isopropylidene acetal group of disaccharide **8** by treatment with aqueous acetic acid at 70 °C, without affecting levulinoyl, acetyl, benzyl, *p*-bromobenzyl, benzyloxycarbonyl and methyl carboxylate groups, to afford the corresponding 7,8-vicinal diol **32** (Scheme 5). Regio- and stereoselective glycosylation of diol **32** with Ara4N building block to trisaccharide **33** proved challenging. Thus, leaving group, catalyst, temperature and solvent effects were optimized to obtain a satisfactory selectivity. When 1.6 equiv. Ara4N *N*-phenyl trifluoroacetimidate **17**[13d] was used, glycosylation with disaccharide **32** activated by TMSOTf in a mixture of CH_2Cl_2/Et_2O at −78 °C provided the desired β-(1→8) linked trisaccharide **33** in 58% yield as the major product, albeit accompanied with traces of the anomeric stereoisomer and regioisomers as well as the 7,8-di-Ara4N byproduct. The anomeric configuration of the Ara4N residue in **33** was assigned as a 1,2-*cis*-β-glycosidic linkage based on the coupling constant between *H*-1′ and *H*-2′ of Ara4N ($J_{H\text{-}1',H\text{-}2'}$ = 3.2 Hz, $\delta_{H\text{-}1'}$ = 4.88 ppm).[26] Acetylation of the 7-hydroxyl group in **33** with acetic anhydride in CH_2Cl_2 gave trisaccharide **34** in 83% yield. The attachment site of Ara4N in **33** was confirmed by the low-field chemical shift of 7″-H of Kdo residue in **34** due to the *O*-7″ acetyl moiety ($\delta_{H\text{-}7''}$ = 5.03 ppm).[26] Unmasking of **34** afforded trisaccharide **35** in 82% yield using hydrazine and acetic acid. A TMSOTf-catalyzed heptosylation of trisaccharide **35** with heptose building block **12** afforded tetrasaccharide **36** in 81% yield as the α-anomer. Removal of all ester groups in **36** with aqueous sodium hydroxide, followed by hydrogenolysis of the azide, benzyl, PBB and benzyloxycarbonyl groups over Pd/C in a mixture of methanol, water and acetic acid, furnished target tetrasaccharide **7** in 80% yield over two steps. The configuration of tetrasaccharide **7** was confirmed by the coupling constants between *C*-1 and *H*-1 of the corresponding heptoses and Ara4N ($^1J_{C-H}$ = 174.6, 171.0 and 171.0 Hz, respectively).[26] The amine of the aminopentyl linker in **7** is more reactive than the amine of 4-amino-4-deoxy-L-Ara4N moiety,[31] therefore the amine of the artificial linker of **7** will be selectively conjugated to a carrier protein.

Monosaccharide **1** and disaccharide **3** were prepared by standard global deprotection of the corresponding mono- and disaccharide skeleton (see Supporting Information for details, SI). Monosaccharide **2** was prepared by attachment of Hep **11** to the *N*-benzyl-*N*-benzyloxycarbonyl pentyl linker followed by global deprotection (see SI for details).

The ^{1}H NMR spectra, especially the chemical shifts of the anomeric protons, of synthetic antigens **4**−**7** were found to be nearly identical to those reported for isolated LPS fragments (Figure 3).[32−34] In addition, assignment of the NMR signals of **1**−**7** confirmed the structural integrity of the desired oligosaccharides (see SI for details). The synthetic antigens **1**−**7** of LPS, equipped with a pentyl amine linker at the reducing end, were obtained for immunological studies.

■ CONCLUSION

Here, we describe a flexible synthetic strategy for the diversity-oriented synthesis of branched LPS core oligosaccharides. This novel approach provided access to densely sterically crowded oligosaccharides such as common core trisaccharide **4** found in most Gram-negative bacteria and the conserved tetrasaccharide **5**−**7** in *Y. pestis*, *H. influenzae* and *Proteus*. The diversity-oriented approach relied on the recruitment of a common orthogonal protected disaccharide **8** that was extended with various flexible modules including unique Hep and Ara4N monosaccharides and branched Hep-Hep disaccharides. The regio- and stereoselective extension of the Kdo 7,8-diol with Ara4N proved challenging but was accomplished by identification of appropriate reaction conditions. This synthetic strategy is modular and flexible so as to access other LPS oligosaccharides consisting of Hep and Kdo rapidly. These diverse LPS core oligosaccharides, with a pentyl amine linker at the reducing end, are currently undergoing immunological assessment.

■ EXPERIMENTAL SECTION

Reagents and General Procedures. Commercial reagents were used without further purification except where noted. Solvents were dried and redistilled prior to use in the usual way. All reactions were performed in oven-dried glassware with magnetic stirring under an inert atmosphere unless noted otherwise. Analytical thin layer chromatography (TLC) was performed on Kieselgel 60 F254 glass plates precoated with a 0.25 mm thickness of silica gel. The TLC plates were visualized with UV light and by staining with Hanessian solution (ceric sulfate and ammonium molybdate in aqueous sulfuric acid) or sulfuric acid-ethanol solution. Column chromatography was performed on Fluka Kieselgel 60 (230−400 mesh). Optical rotations (OR) were measured with a Schmidt & Haensch UniPol L1000 polarimeter at a concentration (c) expressed in g/100 mL. ^{1}H and ^{13}C NMR spectra were measured with a Varian 400-MR or Varian 600-MR spectrometer with Me_4Si as the internal standard. Multiplicities are quoted as singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), triplet (t), or multiplet (m). Spectra were assigned using COSY and HSQC. All NMR chemical shifts (δ) were recorded in ppm and coupling constants (J) were reported in Hz. High-resolution ESI and MALDI mass spectra were recorded on an IonSpec Ultra instrument. MALDI-TOF spectra were recorded on a Bruker Daltonics Autoflex Speed, using 2,4,6-trihydroxyacetophenone (THAP) as the matrix.

Methyl [*N*-Benzyl−benzyloxycarbonyl-5-aminopentyl (2-*O*-acetyl-3,7-di-*O*-benzoyl-4-*O*-*para*-bromobenzyl-6-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1→7)-(2,6-di-*O*-benzyl-3-*O*-levulinoyl-4-*O*-*para*-bromobenzyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1→3)-(2,7-di-*O*-acetyl-4-*O*-*para*-bromobenzyl-6-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1→5)-4-*O*-benzyl-7,8-*O*-isopropylidene-3-deoxy-α-D-*manno*-oct-2-ulopyranosid]onate (26). To a stirred mixture of building block **25** (42 mg, 27 μmol), building block **20** (23 mg, 19 μmol), and freshly activated 4 Å MS in anhydrous diethyl ether and dichloromethane (1/1, v/v, 2 mL) at 0 °C, was added TMSOTf in CH_2Cl_2 (0.05 M, 56 μL) under nitrogen. The temperarture was allowed to warm to room temperature and the stirring continued for 1 h. The mixture was quenched with Et_3N, and filtered. The filtrate was concentrated *in vacuo* to give a residue that was purified by silica gel column chromatography (toluene/EtOAc: 7/1) to afford **26** (28 mg, 67%) as a white solid: $[\alpha]^{20}_D$ = +15.9 (*c* 0.25, $CHCl_3$); ^{1}H NMR (600 MHz, Pyridine-d_5) δ 8.25−7.21 (m, 57 H, Ar), 6.34 (dd, J = 3.0, 10.2 Hz, 1 H, H-3), 6.11 (br s, 1 H, H-2), 5.92 (br s, 1 H, H-1″), 5.88 (br s, 1 H, H-1), 5.81 (br s, 1 H, H-2″), 5.76 (dd, J = 3.0, 7.2 Hz, 1 H, H-3′), 5.63 (br s, 1 H, H-1′), 5.62 (d-like, J = 12.0 Hz, 1 H), 5.41−5.31 (m, 3 H),

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5.17 (m, 1 H), 5.08−4.68 (m, 27 H), 4.64−4.54 (m, 5 H), 4.45 (m, 1 H), 4.36 (d-like, J = 11.4 Hz, 1 H), 4.25 (m, 2 H), 4.20 (t, J = 3.0 Hz, 1 H), 4.00 (d-like, J = 8.4 Hz, 1 H), 3.82 (s, 3 H, C(O)OCH_3), 3.60 (m, 2 H), 3.43 (m, 1 H), 3.32 (m, 1 H), 2.69 (m, 2 H, C(O)CH_2CH_2C(O)), 2.63 (m, 2 H, C(O)CH_2CH_2C(O)), 2.52 (m, 1 H, H-3‴*e*), 2.35 (t, J = 12.0 Hz, 1 H, H-3‴*a*), 2.27 (s, 3 H, C(O)CH_3), 2.01 (s, 3 H, C(O)CH_3), 1.98 (s, 3 H, C(O)CH_3), 1.96 (s, 3 H, C(O)CH_3), 1.65 (m, 4 H, CCH_2C), 1.42 (s, 3 H, C(CH_3)$_2$), 1.39 (s, 3 H, C(CH_3)$_2$), 1.29 (m, 2 H, CCH_2C); ^{13}C NMR (150 MHz, Pyridine-d_5) δ 206.8, 173.2, 171.3, 171.1, 170.2, 169.6, 166.9, 166.6, 150.9, 140.7, 140.5, 140.1, 139.6, 139.5, 139.3, 139.1, 138.9, 138.7, 136.4, 135.8, 134.3, 134.0, 132.4, 132.3, 131.4, 130.9, 130.7, 130.6, 130.5, 130.2, 129.8, 129.7, 129.6, 129.5, 129.4, 129.3, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 124.4, 123.8, 122.3, 122.0, 110.7 (C(CH_3)$_2$), 102.1 (C-1′, $J_{C,H}$ = 174.0 Hz), 100.9 (C-2‴), 98.1 (C-1, $J_{C,H}$ = 179.4 Hz), 97.9 (C-1″, $J_{C,H}$ = 175.8 Hz), 79.7, 77.9, 77.3, 76.8, 76.2, 76.0, 75.7, 75.3, 74.9, 74.8, 74.5, 74.2, 74.1, 74.0, 73.9, 73.8, 73.7, 73.6, 73.5, 73.3, 72.4, 72.1, 71.7, 71.3, 71.2, 68.7, 67.8, 67.6, 67.5, 64.7, 53.4, 38.5, 33.4 (C-3‴), 30.6, 30.4, 30.1, 29.2, 27.8, 25.7, 24.4, 21.5, 21.4, 21.3; HRMS (ESI) m/z calcd for $C_{134}H_{144}Br_3NO_{35}Na$ $[M + Na]^+$ 2589.6980, found 2589.7470.

Methyl [*N*-Benzyl−benzyloxycarbonyl-5-aminopentyl (2,3,7-tri-*O*-acetyl-4-*O*-*para*-bromobenzyl-6-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1→2)-(3,7-di-*O*-benzoyl-4-*O*-*para*-bromo-benzyl-6-*O*-benzyl-L-*glycero*-α-D-*manno*-hepto-pyranosyl)-(1→3)-(2,7-di-*O*-acetyl-4-*O*-*para*-bromobenzyl-6-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1→5)-4-*O*-benzyl-7,8-*O*-isopropylidene-3-deoxy-α-D-*manno*-oct-2-ulopyranosid]onate (31). To a stirred mixture of building block **30** (45 mg, 0.032 mmol), building block **20** (23 mg, 0.019 mmol), and freshly activated 4 Å MS in anhydrous diethyl ether and dichloromethane (1/1, v/v, 2.8 mL) at 0 °C, was added TMSOTf in CH_2Cl_2 (0.05 M, 64 μL) under nitrogen. The temperature was allowed to warm to room temperature and the stirring continued for 1 h. The mixture was quenched with Et_3N, and filtered. The filtrate was concentrated *in vacuo* to give a residue that was purified by silica gel column chromatography (toluene/EtOAc: 10/1 to 8/1) to afford **31** (30 mg, 65%) as a white foam: $[\alpha]^{20}_D$ = +10.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, Pyridine-d_5) δ 8.54−6.87 (m, 52 H, Ar), 6.15 (br s, 1 H, H-1′), 6.10 (dd, J = 3.0, 9.6 Hz, 1 H), 6.05 (m, 2 H), 5.82 (br s, 1 H, H-1), 5.69 (t-like, J = 2.4 Hz, 1 H), 5.46 (dd, J = 4.2, 10.8 Hz, 1 H), 5.39 (m, 2 H), 5.11−4.86 (m, 24 H), 4.81−4.68 (m, 4 H), 4.62−4.48 (m, 5 H), 4.34 (m, 2 H), 4.23 (m, 1 H), 4.00 (m, 1 H), 3.96 (s, 3 H, C(O)OCH_3), 3.82 (dd, J = 6.0, 8.4 Hz, 1 H), 3.74 (m, 1 H), 3.50−3.44 (m, 3 H), 3.30 (m, 1 H), 2.76 (m, 1 H, H-3″*e*), 2.53 (t, J = 10.8 Hz, 1 H, H-3″*a*), 2.23 (s, 3 H, C(O)CH_3), 2.14 (s, 6 H, C(O)CH_3), 2.13 (s, 3 H, C(O)CH_3), 1.98 (s, 3 H, C(O)CH_3), 1.58 (m, 4 H, CCH_2C), 1.41 (s, 3 H, C(CH_3)$_2$), 1.34 (s, 3 H, C(CH_3)$_2$), 1.29 (m, 2 H, CCH_2C); ^{13}C NMR (150 MHz, Pyridine-d_5) δ171.9, 171.8, 171.7, 171.6, 171.2, 170.0, 168.0, 167.0, 151.5, 150.7, 141.1, 141.0, 140.2, 140.1, 139.9, 139.6, 139.5, 139.2, 137.0, 136.4, 134.9, 134.7, 133.1, 133.0, 132.5, 132.1, 131.7, 131.6, 131.4, 130.9, 130.6, 130.5, 130.4, 130.3, 130.2, 130.1, 130.0, 129.9, 129.6, 129.5, 129.4, 129.3, 129.2, 129.0, 128.9, 128.8, 125.0, 124.4, 122.9, 122.7, 122.3, 111.1 (C(CH_3)$_2$), 100.9 (C-1′, $J_{C,H}$ = 176.0 Hz), 100.7 (C-2″), 98.9 (C-1, $J_{C,H}$ = 176.4 Hz), 80.3, 77.4, 76.8, 76.5, 76.0, 75.6, 75.3, 75.1, 74.9, 74.8, 74.3, 74.2, 74.1, 74.0, 73.9, 73.7, 73.4, 73.1, 72.9, 71.7, 71.6, 69.3, 68.4, 68.2, 66.6, 65.1, 64.2, 53.8, 33.4 (C-3″), 31.2, 30.8, 28.2, 26.5, 24.9, 22.3, 22.2, 22.1, 22.0, 21.8; HRMS (MALDI) m/z calcd for $C_{126}H_{136}Br_3NO_{35}Na$ $[M + Na]^+$ 2485.6354, found 2485.5990.

Methyl 2,7-Di-*O*-acetyl-3-*O*-levulinoyl-4-*O*-*para*-bromobenzyl-6-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→5)-[4-azido-2,3-di-*O*-benzyl-4-deoxy-β-L-arabinopyranosyl-(1→8)]-(*N*-benzyl−benzyloxycarbonyl-5-aminopentyl 7-*O*-acetyl-4-*O*-benzyl-3-deoxy-α-D-*manno*-oct-2-ulopyranoside)onate (34). To a stirred mixture of building block **17** (130 mg, 0.247 mmol), building block **32** (190 mg, 0.148 mmol), and freshly activated 4 Å MS in anhydrous diethyl ether and dichloromethane (1/1, v/v, 20 mL) at −78 °C, was added TMSOTf in CH_2Cl_2 (0.05 M, 164 μL) under nitrogen. The temperarture was allowed to warm to room temperature and the stirring continued for 2 h. The mixture was quenched with Et_3N, and filtered. The filtrate was concentrated *in vacuo* to give a residue that was purified by silica gel column chromatography (hexane/EtOAc: 6/5 to 1/1) to afford **33** (138 mg, 58%) as a white foam: $[\alpha]^{20}_D$ = +59.6 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45−7.07 (m, 34 H), 5.38 (dd, J = 3.2, 9.6 Hz, 1 H, H-3), 5.30 (br s, 1 H, H-2), 5.24 (br s, 1 H, H-1), 5.16 (m, 2 H, BnCH_2OC(O)N), 4.88 (d, J = 3.2 Hz, 1 H, H-1′), 4.80 (m, 2 H), 4.72−4.61 (m, 4 H), 4.54−4.37 (m, 7 H), 4.30 (m, 1 H), 4.20 (d-like, J = 11.6 Hz, 1 H), 4.12 (m, 1 H), 4.05−3.94 (m, 4 H), 3.87−3.80 (m, 4 H), 3.75 (s, 3 H, C(O)OCH_3), 3.71 (m, 1 H), 3.57 (m, 2 H), 3.39 (m, 2 H), 3.21 (m, 3 H), 2.73−2.57 (m, 2 H, C(O)CH_2CH_2C(O)), 2.43 (m, 2 H, C(O)CH_2CH_2C(O)), 2.27 (m, 1 H, H-3″*e*), 2.13 (s, 3 H), 2.08 (s, 3 H), 2.00 (m, 1 H, H-3″*a*), 1.97 (s, 3 H), 1.46 (m, 4 H), 1.21 (m, 2 H); HRMS (ESI) m/z calcd for $C_{85}H_{97}BrN_4O_{23}Na$ $[M + Na]^+$ 1643.5625, found 1643.5727. To a solution of trisaccharide **33** (100 mg, 0.062 mmol) and DMAP (60 mg, 0.491 mmol) in CH_2Cl_2 (9 mL), was added Ac_2O (0.6 mL). After being stirred at room temperature overnight, the mixture was washed with saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/EtOAc: 1/1) to give **34** (85 mg, 83%) as a colorless syrup: $[\alpha]^{20}_D$ = +56.5 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43−7.04 (m, 34 H), 5.37 (dd, J = 3.2, 9.6 Hz, 1 H, H-3), 5.23 (br s, 1 H, H-2), 5.14 (m, 2 H, BnCH_2OC(O)N), 5.03 (m, 1 H, H-7″), 4.86 (d, J = 3.2 Hz, 1 H, H-1′), 4.81 (d, J = 1.2 Hz, 1 H, H-1), 4.75 (d-like, J = 12.0 Hz, 1 H, OCH_2Ar), 4.69−4.60 (m, 4 H), 4.53−4.33 (m, 7 H), 4.22−3.87 (m, 11 H), 3.76 (m, 3 H), 3.74 (s, 3 H, C(O)OCH_3), 3.54 (m, 1 H), 3.47 (m, 1 H), 3.19−3.12 (m, 3 H), 2.76 (m, 1 H, C(O)CH_2CH_2C(O)), 2.60 (m, 1 H, C(O)CH_2CH_2C(O)), 2.43 (m, 2 H, C(O)CH_2CH_2C(O)), 2.26 (m, 1 H, H-3″*e*), 2.13 (s, 3 H), 2.10 (s, 3 H), 2.00 (m, 1 H, H-3″*a*), 1.98 (s, 3 H), 1.94 (s, 3 H), 1.42 (m, 4 H), 1.21 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.4, 171.5, 170.6, 170.0, 169.7, 168.3, 156.6, 156.1, 138.4, 137.9, 137.7, 137.4, 136.7, 131.3, 129.1, 129.0, 128.8, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.2, 127.1, 121.3, 98.8 (C-2″), 98.3 (C-1), 98.1 (C-1′), 77.3, 75.9, 73.3, 73.2, 72.9, 72.8, 72.7, 72.4, 72.1, 71.9, 70.4, 70.3, 70.0, 67.1, 65.3, 65.1, 63.5, 60.7, 59.6, 52.4, 50.3, 50.0, 47.0, 46.1, 37.8, 31.9 (C-3″), 29.8, 29.7, 29.3, 27.9, 27.5, 23.3, 21.1, 21.0; HRMS (ESI) m/z calcd for $C_{87}H_{99}BrN_4O_{24}Na$ $[M + Na]^+$ 1685.5730, found 1685.5767.

Methyl 2-*O*-Acetyl-3,7-di-*O*-benzoyl-4-*O*-*para*-bromobenzyl-6-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)-2,7-di-*O*-acetyl-4-*O*-*para*-bromobenzyl-6-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→5)-[4-azido-2,3-di-*O*-benzyl-4-deoxy-β-L-arabinopyranosyl-(1→8)]-(*N*-benzyl−benzyloxycarbonyl-5-aminopentyl 7-*O*-acetyl-4-*O*-benzyl-3-deoxy-α-D-*manno*-oct-2-ulopyranoside)onate (36). To a stirred mixture of building block **12** (34 mg, 38 μmol), building block **35** (30 mg, 19 μmol), and freshly activated 4 Å MS in anhydrous dichloromethane (3 mL) at 0 °C, was added TMSOTf in CH_2Cl_2 (0.05 M, 62 μL) under nitrogen. The temperature was allowed to warm to room temperature and the stirring continued for 1 h. The mixture was quenched with Et_3N, and filtered. The filtrate was concentrated *in vacuo* to give a residue that was purified by silica gel column chromatography (hexane/EtOAc: 5/2 to 2/1) to afford **36** (35 mg, 81%) as a white foam: $[\alpha]^{20}_D$ = +24.5 (*c* 0.3, $CHCl_3$); ^{1}H NMR (400 MHz, Pyridine-d_5) δ 8.46−7.10 (m, 53 H), 6.15 (dd, J = 3.2, 9.6 Hz, 1 H, H-3), 5.97 (br s, 1 H, H-2′), 5.93 (m, 1 H, H-2), 5.69 (s, 1 H, H-1), 5.50 (m, 1 H, H-7‴), 5.48 (m, 1 H), 5.42 (s, 1 H, H-1′), 5.36 (m, 2 H, BnCH_2OC(O)N), 5.29 (d-like, J = 11.6 Hz, 1 H), 5.26 (d, J = 3.2 Hz, 1 H, H-1″), 5.13 (m, 1 H), 5.05−4.96 (m, 10 H), 4.86−4.72 (m, 8 H), 4.70−4.59 (m, 5 H), 4.53 (m, 1 H), 4.47 (m, 2 H), 4.38 (m, 1 H), 4.24 (m, 2 H), 4.15−4.04 (m, 3 H), 3.93 (m, 1 H), 3.84 (m, 1 H), 3.79 (s, 3 H, C(O)OCH_3), 3.68 (m, 1 H), 3.49 (m, 1 H), 3.37 (m, 1 H), 3.27 (m, 1 H), 2.72 (d-like, J = 10.0 Hz, 1 H, H-3‴*e*), 2.50 (t, J = 12.4 Hz, 1 H, H-3‴*a*), 2.17 (s, 3 H), 2.14 (s, 3 H), 2.05 (s, 3 H), 1.99 (s, 3 H), 1.60 (m, 4 H), 1.36 (m, 2 H); ^{13}C NMR (100 MHz, Pyridine-d_5) δ 170.8, 170.5, 170.3, 170.0, 168.8, 166.9, 165.9, 139.6, 139.4, 139.3, 139.1, 138.9, 138.7, 138.3, 138.1, 136.1, 135.1, 133.8, 133.5, 131.8, 131.6, 130.9, 130.4, 130.3, 130.0, 129.8, 129.7, 129.5, 129.3, 129.0,

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128.9, 128.8, 128.5, 128.3, 128.2, 128.1, 127.9, 127.6, 125.8, 124.1, 123.7, 123.0, 121.6, 121.5, 99.8 (C-2‴), 99.6 (C-1, $J_{C,H}$ = 176.0 Hz), 98.9 (C-1′, $J_{C,H}$ = 174.8 Hz), 98.7 (C-1″, $J_{C,H}$ = 170.4 Hz), 79.9, 77.8, 77.1, 76.7, 76.2, 75.9, 75.5, 74.2, 74.0, 73.9, 73.3, 73.1, 72.9, 72.8, 72.4, 72.3, 72.2, 71.3, 70.8, 70.7, 70.4, 67.2, 66.0, 64.8, 64.7, 64.1, 61.0, 60.4, 52.5, 50.7, 50.3, 47.5, 46.7, 32.7 (C-3‴), 30.1, 29.9, 28.6, 28.0, 23.8, 21.2, 21.0, 20.8, 20.5; HRMS (ESI) *m*/*z* calcd for $C_{119}H_{126}Br_2N_4O_{31}Na$ $[M + Na]^+$ 2290.6684, found 2290.6683.

L-*Glycero*-α-D-*manno*-heptopyranosyl-(1→7)-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→5)-2-(5-amino)pentyl-3-deoxy-α-D-*manno*-oct-2-ulopyra nosidonic acid (5). A solution of tetrasaccharide **26** (20 mg, 7.79 μmol) in acetic acid/water (8/1, v/v, 2.7 mL) was stirred at 70 °C overnight until TLC indicated complete conversion of starting material. The mixture was coevaporated with toluene and dried *in vacuo* to give the corresponding diol as a white solid. The resulting diol was dissolved in a mixture of dioxane, methanol and 1 M aq NaOH (3/1/1, v/v/v, 2.00 mL). After stirring at room temperature overnight, the reaction mixture was diluted with methanol and neutralized with Amberlite IR120 H^+ resin. After filtration, the filtrate was concentrated *in vacuo* and eluted through Sephadex LH-20 column (CH_2Cl_2/MeOH: 1/1) to give the corresponding tetrasaccharide as a white solid. A mixture of the resulting tetrasaccharide and Pd/C (100 mg, 10%) in methanol, water and acetic acid (65/13/1, v/v/v, 4.86 mL) was stirred under an atmosphere of H_2 at room temperature for 24 h. Filtration, concentration *in vacuo* and elution through Sephadex LH-20 column (H_2O) provided **5** (5 mg, 71% over three steps) as a white solid: $[\alpha]^{20}_D$ = +83.4 (*c* 0.3, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 5.13 (s, 1 H, H-1″), 5.11 (s, 1 H, H-1′), 4.98 (s, 1 H, H-1), 4.17−4.12 (m, 4 H, H-4‴), 4.06−3.95 (m, 8 H), 3.91−3.62 (m, 15 H), 3.41 (m, 1 H, OCH_2), 3.29 (m, 1 H, OCH_2), 3.00 (t, *J* = 7.8 Hz, 2 H, NCH_2), 2.07 (dd, *J* = 4.2, 12.6 Hz, 1 H, H-3‴*e*), 1.83 (t, *J* = 12.6 Hz, 1 H, H-3‴*a*), 1.68 (m, 2 H, CCH_2C), 1.61 (m, 2 H, CCH_2C), 1.42 (m, 2 H, CCH_2C); ^{13}C NMR (150 MHz, D_2O) δ 177.7 (C(O)), 105.2 (C-1″, $J_{C,H}$ = 175.8 Hz), 103.9 (C-1′, $J_{C,H}$ = 173.4 Hz), 103.1 (C-1, $J_{C,H}$ = 172.2 Hz), 102.5 (C-2‴), 82.7, 77.7, 75.2, 74.7, 74.1, 73.9, 73.4, 73.1, 72.7, 72.6, 72.5, 72.1, 71.9, 71.5, 71.4, 71.0, 69.0, 68.8, 68.4, 67.8, 66.0, 65.7, 65.6, 65.5, 42.0 (NCH_2), 37.6 (C-3‴), 30.8, 29.1, 25.0; HRMS (ESI) *m*/*z* calcd for $C_{34}H_{61}NO_{26}Na$ $[M + Na]^+$ 922.3380, found 922.3325.

L-*Glycero*-α-D-*manno*-heptopyranosyl-(1→2)-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→5)-2-(5-amino)pentyl-3-deoxy-α-D-*manno*-oct-2-ulopyra nosidonic acid (6). A solution of tetrasaccharide **31** (11 mg, 4.46 μmol) in acetic acid/water (8/1, v/v, 1.3 mL) was stirred at 70 °C for 7 h until TLC indicated complete conversion of starting material. The mixture was coevaporated with toluene and dried *in vacuo* to give the corresponding diol as a white solid. The resulting diol was dissolved in a mixture of dioxane, methanol and 1 M aq NaOH (3/1/1, v/v/v, 2.0 mL). After stirring at room temperature overnight, the reaction mixture was diluted with methanol and neutralized with Amberlite IR120 H^+ resin. After filtration, the filtrate was concentrated *in vacuo* and eluted through Sephadex LH-20 column (CH_2Cl_2/MeOH: 1/1) to give the corresponding tetrasaccharide as a white solid. A mixture of the resulting tetrasaccharide and Pd/C (100 mg, 10%) in methanol, water and acetic acid (68/17/1, v/v/v, 5.06 mL) was stirred under an atmosphere of H_2 at room temperature for 24 h. Filtration, concentration *in vacuo* and elution through Sephadex LH-20 column (H_2O) provided **6** (3 mg, 75% over three steps) as a white solid: $[\alpha]^{20}_D$ = +47.2 (*c* 0.3, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 5.40 (d, *J* = 1.2 Hz, 1 H, H-1′), 5.10 (d, *J* = 1.2 Hz, H-1), 5.08 (d, *J* = 1.8 Hz, H-1″), 4.19−4.15 (m, 2 H, H-4‴), 4.11−3.94 (m, 12 H), 3.91−3.85 (m, 2 H), 3.82 (m, 1 H), 3.78−3.63 (m, 10 H), 3.44 (m, 1 H, OCH_2), 3.30 (m, 1 H, OCH_2), 3.02 (t, *J* = 7.2 Hz, 2 H, NCH_2), 2.12 (dd, *J* = 4.2, 12.6 Hz, 1 H, H-3‴*e*), 1.86 (t, *J* = 12.6 Hz, 1 H, H-3‴*a*), 1.70 (m, 2 H, CCH_2C), 1.63 (m, 2 H, CCH_2C), 1.45 (m, 2 H, CCH_2C); ^{13}C NMR (150 MHz, D_2O) δ 177.7 (C(O)), 104.7 (C-1″, $J_{C,H}$ = 173.4 Hz), 103.9 (C-1, $J_{C,H}$ = 172.2 Hz), 102.8 (C-1′, $J_{C,H}$ = 175.8 Hz), 102.4 (C-2‴), 80.8, 79.7, 77.6, 74.6, 74.4, 74.0, 73.0, 72.7, 72.6, 71.9, 71.7, 71.4, 71.1, 68.9, 68.8, 68.4, 68.3, 65.9, 65.8, 65.6, 65.5, 42.0 (NCH_2), 37.5 (C-3‴), 30.8, 29.1, 25.0; HRMS (MALDI) *m*/*z* calcd for $C_{34}H_{61}NO_{26}Na$ $[M + Na]^+$ 922.3380, found 922.3330.

L-*Glycero*-α-D-*manno*-heptopyranosyl-(1→3)-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→5)-[4-amino-4-deoxy-β-L-arabino-pyranosyl-(1→8)]-2-(5-amino)pentyl-3-deoxy-α-D-*manno*-oct-2-ulopy ranosidonic acid (7). Tetrasaccharide **36** (33 mg, 0.015 mmol) was dissolved in a mixture of dioxane, methanol and 1 M aq NaOH (3/1/1, v/v/v, 2.0 mL). After stirring at room temperature overnight, the reaction mixture was diluted with methanol and neutralized with Amberlite IR120 H^+ resin. After filtration, the filtrate was concentrated *in vacuo* and eluted through Sephadex LH-20 column (CH_2Cl_2/MeOH: 1/1) to give the corresponding tetrasaccharide as a white solid. A mixture of the resulting tetrasaccharide and Pd/C (100 mg, 10%) in methanol, water and acetic acid (85/17/1, v/v/v, 6.06 mL) was stirred under an atmosphere of H_2 at room temperature overnight. Filtration, concentration *in vacuo* and elution through Sephadex LH-20 column (H_2O) provided **7** (10 mg, 80% over two steps) as a white solid: $[\alpha]^{20}_D$ = +87.4 (*c* 0.5, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 5.24 (s, 1 H, H-1), 5.10 (s, 1 H, H-1′), 5.08 (s, 1 H, H-1″), 4.17 (m, 2 H, H-4‴), 4.13−3.89 (m, 13 H), 3.81−3.68 (m, 9 H), 3.60 (d-like, *J* = 9.6 Hz, 1 H), 3.39 (m, 1 H, OCH_2), 3.33 (m, 1 H, OCH_2), 3.03 (t, *J* = 7.8 Hz, 2 H, NCH_2), 2.11 (dd, *J* = 4.2, 13.2 Hz, 1 H, H-3‴*e*), 1.87 (t, *J* = 12.6 Hz, 1 H, H-3‴*a*), 1.70 (m, 2 H, CCH_2C), 1.62 (m, 2 H, CCH_2C), 1.44 (m, 2 H, CCH_2C); ^{13}C NMR (150 MHz, D_2O) δ 177.6 (C(O)), 104.3 (C-1, $J_{C,H}$ = 174.6 Hz), 104.0 (C-1′, $J_{C,H}$ = 171.0 Hz), 102.5 (C-2‴), 101.4 (C-1″, $J_{C,H}$ = 171.0 Hz), 79.0, 77.4, 74.6, 74.4, 73.3, 72.7, 71.5, 71.2, 70.4, 68.7, 68.2, 65.8, 60.8, 54.4, 42.0 (NCH_2), 37.4 (C-3‴), 30.9, 29.2, 25.2; HRMS (ESI) *m*/*z* calcd for $C_{32}H_{57}N_2O_{23}$ $[M - H]^-$ 837.3352, found 837.3374.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, characterization data and copies of NMR spectra for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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