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Stereocontrolled Synthesis of the D- and L-*glycero*-β-D-*manno*-Heptopyranosides and Their 6-Deoxy Analogues. Synthesis of Methyl α-L-*Rhamno*-pyranosyl-(1→3)-D-*glycero*-β-D-*manno*-heptopyranosyl-(1→3)-6-deoxy-*glycero*-β-D-*manno*-heptopyranosyl-(1→4)-α-L-*rhamno*-pyranoside, a Tetrasaccharide Subunit of the Lipopolysaccharide from *Plesimonas shigelloides*

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Abstract: The synthesis of D- and L-*glycero*-α-*manno*-thioheptopyranosides, protected with 4,6-*O*-alkylidene-type acetals is described. In glycosylations carried out with preactivation with the 1-benzenesulfinylpiperidine/trifluoromethanesulfonic anhydride couple, both the D- and L-*glycero* series exhibit excellent β-selectivity with a range of glycosyl acceptors. In contrast, a 4,7-*O*-alkylidene acetal was found not to afford β-selectivity. With a 4,6-*O*-[1-cyano-2-(2-iodophenyl)ethylidene] acetal protected thioglycoside, excellent β-selectivity was obtained in glycosylation reactions, and subsequent treatment with tributyltin hydride and azoisobutyronitrile brought about clean fragmentation to the 6-deoxy-*glycero*-β-D-*manno*-heptopyranosides. This chemistry was applied to the stereocontrolled synthesis of methyl α-L-*rhamno*-pyranosyl-(1→3)-D-*glycero*-β-D-*manno*-heptopyranosyl-(1→3)-6-deoxy-*glycero*-β-D-*manno*-heptopyranosyl-(1→4)-α-L-*rhamno*-pyranoside, a component of the lipopolysaccharide from *Plesimonas shigelloides*.

Introduction

Structurally complex lipopolysaccharides (LPS) are amphipathic and microheterogeneous glycolipids and essential components of the outer membrane of Gram-negative bacteria.[1] Numerous immunological and pathophysiological effects in bacterial infections are mediated by LPS. Due to the increasing resistance of many bacterial strains against conventional antibiotics, a detailed understanding of the immunological responses is necessary in order to develop novel drugs, which may prevent the assembly of a functionally intact bacterial cell wall by inhibition of its biosynthesis.[2] Thus, well-defined syntheses of complex oligosaccharides and glycoconjugates corresponding to the native bacterial structures are essential, as the synthetic derivatives can be employed to (i) locate immunodominant motifs, (ii) determine the specificity of the antibody response obtained from native structures, and (iii) investigate the processing of glycoproteins by the immune systems. Moreover, such synthetic oligosaccharides could act as vaccine candidates.[3] The L-*glycero*-D-*manno*- and D-*glycero*-D-*manno*-heptoses, are common constituents of both the inner- and outer-core regions of LPS of many pathogenic bacteria, where they are present mostly in the α-anomeric configuration.[2] The synthesis of higher-carbon sugars such as heptoses has been investigated for more than a century,[4] and several methods have been developed.[2,4,5] The synthesis of a large number of complex oligosaccharides containing α-L,D- or α-D,D-heptopyranosides (Hep*p*), has been achieved by Oscarson and co-workers.[3,6]

Plesimonas shigelloides is a Gram-negative, flagellated, rod-shaped bacterium which has been isolated from a variety of sources such as fresh water, surface water, and many wild and domestic animals. The infections correlate strongly with the surface water contamination and are particularly common in tropical and subtropical habitats. The structure of the *O*-specific side chain of the LPS, strain CNCTC 113/92 (serotype 54) has been recently elucidated by a combination of ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry, monosaccharide analysis, and immunological methods.[7] Interestingly, the polysaccharide is

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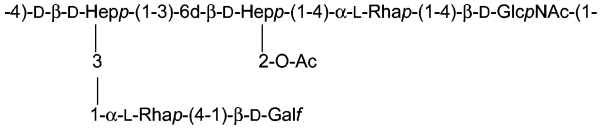


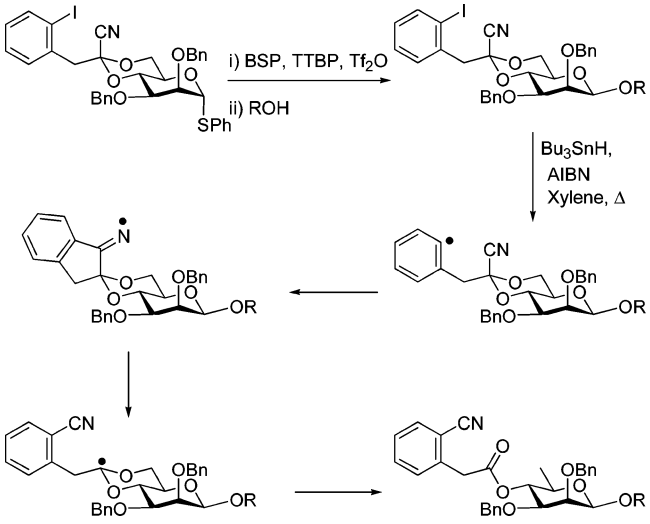
Figure 1. Repeating unit of the *O*-specific polysaccharide from CNCTC 113/92 LPS (serotype 54).

composed of a novel hexasaccharide repeating unit (Figure 1) containing two unusual β-linked heptose units, one of which is 6-deoxy.

These unique structural features of the repeating unit prompted our investigation of stereocontrolled β-glycosylation of the D,D- and L,D-heptoses. The synthetic challenge was heightened by the presence of the 6-deoxy-*glycero*-β-D-*manno*-heptopyranoside. To design a concise synthesis of the hexasaccharide repeating unit, we needed to address two issues: (i) stereoselective β-glycosydation in D,D- and L,D-heptoses and (ii) efficient generation of the 6-deoxy-β-Hep*p* unit with full regiocontrol at the 6-position and stereocontrol at the anomeric position. The first problem is conceptually similar to that of stereocontrolled β-mannosylation, for which we developed a powerful method using 4,6-*O*-benzylidene-blocked thiomannosides,[8a,b] and/or their sulfoxides.[8c] Our interest in these molecules was further spurred by the insight that might be gained into the underlying reasons for the beneficial influence of the 4,6-*O*-benzylidene acetal on the stereocontrolled preparation of β-mannosides. Originally, working in the *gluco*-series, Fraser-Reid and co-workers suggested that *trans*-fused 4,6-*O*-benzylidene protecting groups restrict the flexibility of the pyranose ring, resulting in an oxacarbenium ion intermediate with a computed (PM3) 20° twist in the ideally *syn*-coplanar C5−O5−C1−C2 system.[9] In a subsequent paper, differential solvation was also computed to be of significance in these so-called torsional disarming effects.[10] More recently, Bols and co-workers provided experimental evidence in support of the notion that the disarming effect of the 4,6-*O*-acetal group is mainly due to the locking of the C5−C6 bond in the deactivating *tg*-conformation.[11] We hypothesized that the inclusion of either an extra axial C6 substituent, as in the 4,6-*O*-benzylidene-protected L-*glycero*-D-*manno*-heptoses, or a corresponding equatorial substituent, as in the D-*glycero*-D-*manno* series, would influence both torsional and solvation considerations differently, whereas the *tg* conformation of the C5−C6 bond would be unchanged. Comparisons of either reactivity or stereoselectivity between the two diastereomeric series might therefore differentiate between the two rationales for the 4,6-*O*-benzylidene group effect.

The second issue perhaps is more intriguing and is analogous to the problems associated with the synthesis of β-D-*rhamno*-pyranosides. The problem of the β-D-*rhamno*-pyranosides reduces to one of the regioselective deoxygenation of D-mannose

Scheme 1. 4,6-*O*-[1-Cyano-2-(2-iodophenyl)ethylidene] Benzylidene Radical Fragmentation



derivatives at the 6-position, which is best achieved after glycosylation so as to take advantage of the directing effect of a 4,6-*O*-benzylidene acetal-type protecting group.[12] In view of the susceptibility of benzyl ethers to hydrogen atom abstraction, neither the Hanessian−Hullar[13] reaction nor the related Roberts' protocol[14] for the cleavage of benzylidene acetals are applicable to the type of complex oligosaccharide synthesis envisaged. With this problem in mind, we initially developed the 4,6-*O*-[α-(2-(2-iodophenyl)ethylthiocarbonyl)benzylidene] group as a surrogate for the 4,6-*O*-benzylidene acetal[15a] and applied it in the total synthesis of the LPS *E. hermanii* ATCC 33650/33652.[15b] However, this method suffers from two limitations: the lengthy synthesis of the thiol and the transesterification reaction, required to introduce the thiol ester, which reduces functional group compatibility. An improved second-generation system, the 1-cyano-2-(2-iodophenyl)ethylidene group, was developed and employed successfully in the synthesis of β-D-*rhamno*-pyranosides (Scheme 1).[16] This new 4,6-*O*-benzylidene surrogate can be easily prepared and introduced under mild conditions. Moreover, it is compatible with a wide variety of protecting groups. However, one question remained in the application of this second-generation system to the synthesis of the 6-deoxy-β-Hep*p* unit, namely the regioselectivity of the radical fragmentation, which might potentially give either a 4- or a 6-deoxy system. Nevertheless, based on the understanding of the radical

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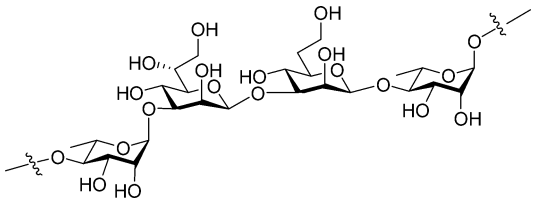


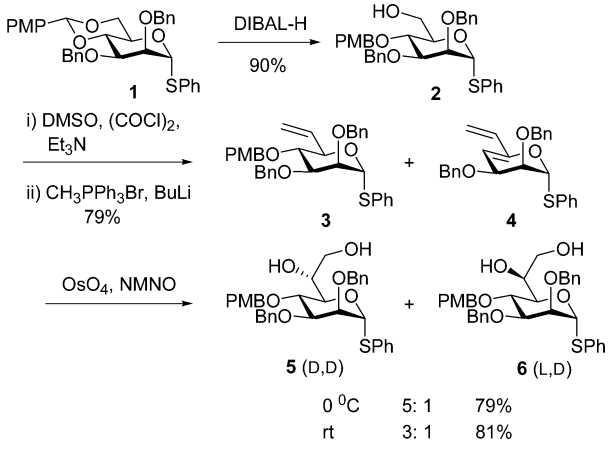
Figure 2. Tetrasaccharide moiety in the repeating unit of the polysaccharide.

fragmentation step and keeping the steric environment in mind, we were confident that the desired 6-deoxy product would be the preferred one. We report here on the successful implementation of this strategy and illustrate it with a concise, fully stereocontrolled synthesis of the tetrasaccharide moeity (Figure 2), containing all the unique and unusual features present in the hexasaccharide repeating unit of the LPS *Plesimonas shigelloides*.

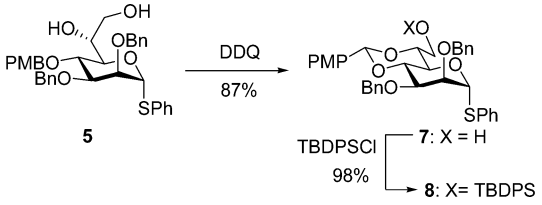
Results and Discussion

Stereoselective Glycosylation in the *glycero*-*manno*-Heptopyranoside Series.[17] The synthesis of a diastereomeric glycosyl donor pair (Scheme 2) began with the known 4,6-*O*-*p*-methoxybenzylidene-protected thiomannoside **1**,[18] which was regioselectively opened to the primary alcohol **2** exclusively in 90% yield with DIBAL-H in dichloromethane.[19] Comparable results were obtained with scandium triflate-catalyzed, BH_3-THF-mediated reduction;[20] all other standard protocols[21] gave mixtures of isomers and considerable cleavage of the acid-labile *p*-methoxybenzyl group. Swern oxidation[22] of **2** gave the corresponding unstable[23] aldehyde, which was carried on to the next step, Wittig olefination,[22] without purification. The yield (65%) of the olefination product **3** was compromised by the concomitant formation of diene **4** in 14% yield.[24] Use of the Nysted reagent[25] for this transformation also generated compound **4**, along with compound **3**, however, in higher yield, whereas the Petasis reagent[26] gave predominantly decomposition products. Treatment of olefin **3** with OsO_4 (5 mol%) and NMNO at 0 °C, and room temperature, furnished diols **5** and **6** in 5:1 and 3:1 diastereomeric ratios and in 79% and 81% yields, respectively (Scheme 2). The stereochemical outcome of this dihydroxylation follows Kishi's empirical rule;[27] the relatively poor diastereoselectivity starting from the sugar with 2,3-*erythro* configuration is also consistent with literature precedent.[5c] Application of the Sharpless asymmetric dihydroxylation protocol was unsatisfactory for this transformation in our hands.[5f,23,28] Compound **5** was converted uneventfully to the D-*glycero*-D-*manno*-heptothioglycoside **8** by oxidative ring closure with DDQ in 87% yield[29] and subsequent silylation of the residual hydroxyl group in **7** with TBDPSCl in 98% yield (Scheme 3). To obtain a significant quantities of the L,D-epimer, the primary hydroxyl group of **5** was silylated, after which Mitsunobu inversion[23] afforded the inverted ester **9** in 92% yield over two steps. Hydrolysis of the ester function then gave the epimeric L,D-heptose **10** in 95% yield (Scheme 4).

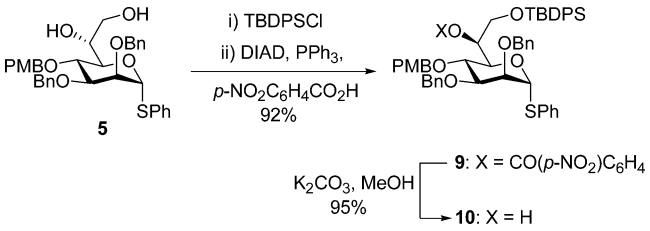
Scheme 2. Preparation of Heptoses **5** and **6**



Scheme 3. Synthesis of D-*glycero*-D-*manno*-Heptothioglycoside



Scheme 4. Inversion of Configuration at C6



Oxidative cyclization of the diol **6** in the L,D-series was difficult due to the developing 1,3-diaxial interaction in the acetal ring. The result was the consistent formation of the hydrolysis product **12** and the less-strained 4,7-*O*-benzylidene aetal **13**, together with the desired acetal **11**. The structures of these acetals (**11** and **13**) were confirmed by transforming the residual hydroxyl groups to the acetate esters (**14** and **15**), when the anticipated chemical shift changes were observed (Scheme 5).

The oxidative cyclization of compound **10** was also comparatively slow, and oxidative cleavage of the *p*-methoxybenzyl

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(18) Crich, D.; Li, W.; Li, H. *J. Am. Chem. Soc.* **2004**, *126*, 15081.
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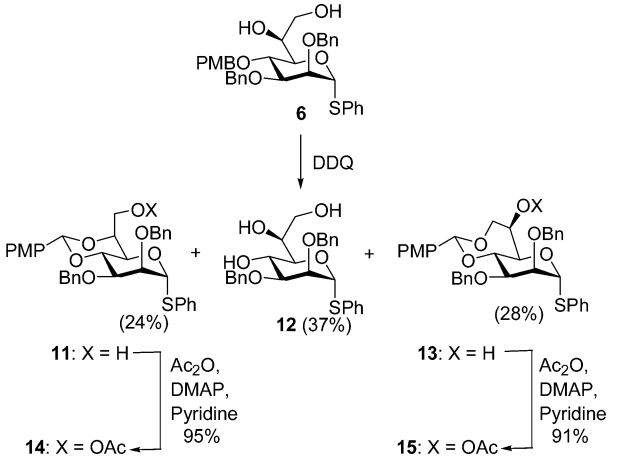
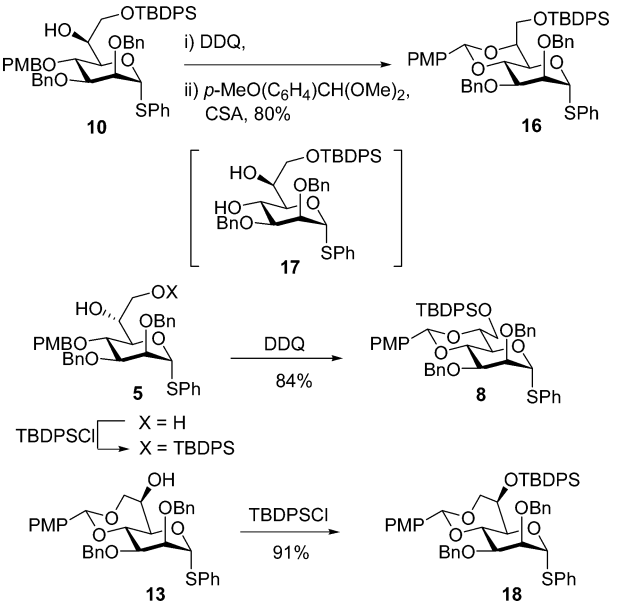
Scheme 5. Oxidative Cyclization of L-*glycero*-D-*manno*-Heptose



group was again a competing reaction producing diol **17** in 48% yield along with the desired L-*glycero*-D-*manno*-heptothioglycoside **16** in 43% yield. In the face of this difficulty, a protocol was devised whereby the reaction mixture from treatment of **10** with DDQ was treated with *p*-methoxybenzaldehyde dimethyl acetal and catalytic camphorsulfonic acid (CSA) to give **16** in 80% yield over two steps (Scheme 6). To investigate the role of a bulky protecting group adjacent to the free hydroxyl group involved in the oxidative cyclization reaction, we silylated the primary hydroxyl group of **5** with TBDPSCl and treated the resulting silyl ether with DDQ. As expected, no significant change in the yield or reaction time was observed and **8** was obtained in 84% yield over two steps (Scheme 6). To investigate the influence of a seven-membered acetal on the anomeric stereoselectivity in the glycosylation reaction, the 4,7-*O*-*p*-methoxybenzylidene acetal **13** was also converted into a glycosyl donor. Thus, **13** was silylated with TBDPSCl to produce **18** in 91% yield (Scheme 6). At this stage, the NOE correlations (Figure 3) served to confirm both the configuration at C6 in compounds **8** and **16** and the equatorial nature of the *p*-methoxyphenyl group in the two acetals.

Scheme 6. Preparation of Glycosyl Donors



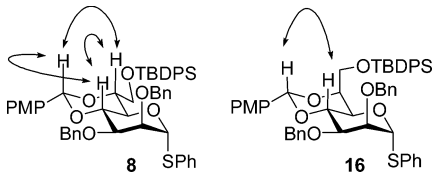
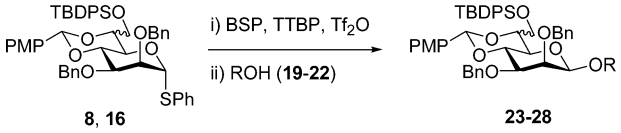


Figure 3. Diagnostic NOE correlations for donors **8** and **16**.

Following our standard thioglycoside activation protocol, a series of glycosylation reactions were then conducted with donors **8** and **16** with the aid of 1-benzenesulfinyl piperidine and trifluoromethanesulfonic anhydride (BSP/Tf_2O)[8a] in the presence of the hindered base 2,4,6-tri-*tert*-butylpyrimidine (TTBP)[30] in dichloromethane at −60 °C (Scheme 7). All couplings were highly β-selective and proceeded in high yields (Table 1). A minor exception to the otherwise exclusive β-selectivity observed with the 4,6-*O*-*p*-methoxybenzylidene protection was the coupling of donor **16** to the acceptor **19** (Table 1, entry 2). This may represent a small difference in reactivity between the two donors, which only becomes apparent with less reactive acceptors such as **19**.[31] Interestingly, the coupling of donor **18**, with its 4,7-*O*-benzylidene acetal, with acceptor **19** yielded the α-disaccharide predominantly (Table 1, entry 7), once again demonstrating the critical influence of the 4,6-*O*-benzylidene protecting group in the stereoselective β-glycosylation. The assignment of anomeric stereochemistry of all the glycosides in Table 1, and all subsequent schemes, was made on the basis of $^1J_{CH}$ coupling constants.[32] Furthermore, in all products arising from donors **8** and **16**, a relatively upfield chemical shift (δ 3.25−3.50 in $CDCl_3$) of the heptose H5 resonance was observed, as is characteristic of 4,6-*O*-benzylidene-protected β-mannosides.[8c]

Scheme 7. Glycosylation with the BSP/Tf_2O Couple

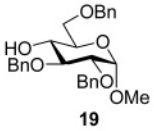
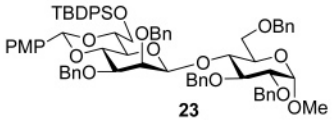
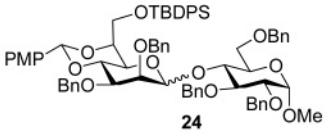
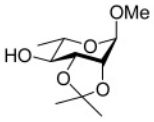
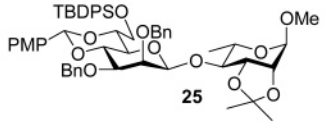
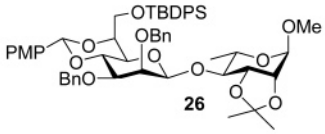
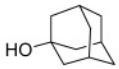
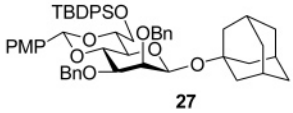
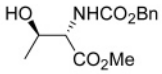
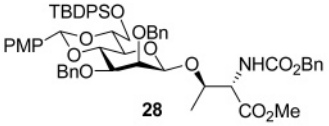
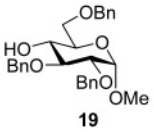
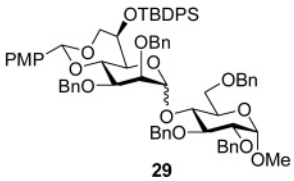


In donor **18**, with its more pliable trioxa[5.4.0]bicycloundecene core, the C5−C6 bond is not held rigidly in the *tg*-conformation but is forced to adopt an approximate *gg*-conformation. Extending the logic of Bols, this reduces its destabilizing influence on any oxacarbenium ion intermediate and leads, ultimately, to the α-selectivity. Alternatively, it can be argued that the greater conformational mobility of this system minimizes any torsional effect on formation of the oxacarbenium ion. Thus, this system alone does not provide a means of differentiating between the two rationales for the benzylidene effect. However, the comparable β-selectivity obtained with donors **8** and **16**, both of which have the rigid *tg*-conformation about the C5−C6 bond but which differ in the position of the C6 substituent, suggests that torsional effects are not the major contributors to the directing effect of the benzylidene acetal.

Turning to deprotection, glycosides **23**, **24**β, and **25** were first exposed to TBAF in THF. This was followed by the removal of the *p*-methoxybenzylidene acetals and the acetonides with

(30) Crich, D.; Smith, M.; Yao, Q.; Picione, J. *Synthesis* **2001**, 323.
(31) Paulsen, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 155.
(32) Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293.

Table 1. Diastereoselective Glycosylation Reaction

Entry	Donor	Acceptor	Product	% Yield (β:α ratio)
1	**8**	 **19**	 **23**	81 (β only)
2	**16**		 **24**	86 (8:1)
3	**8**	 **20**	 **25**	88 (β only)
4	**16**		 **26**	85 (β only)
5	**8**	 **21**	 **27**	88 (β only)
6	**8**	 **22**	 **28**	89 (β only)
7	**18**	 **19**	 **29**	81 (1:4)

catalytic CSA in methanol at reflux. Finally, hydrogenolysis over Pd/C afforded the fully deprotected disaccharides (Table 2).

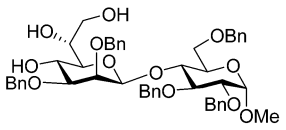
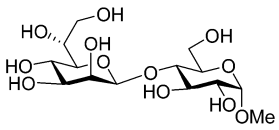
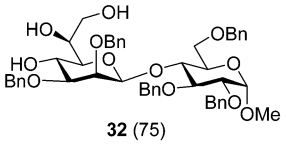
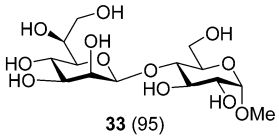
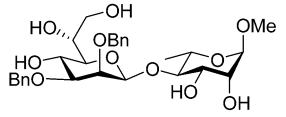
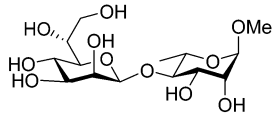
The synthesis of the tetrasaccharide (Figure 2), in principle, can be achieved in a linear fashion by a sequence involving three glycosylation reactions, with intermediate deprotection steps for unmasking the 3-OH group at the nonreducing end, and one radical fragmentation reaction. Two different L-rhamnose building blocks and one common heptose building block are required to achieve the task. Thus, diol **36**[18] was converted to the 3-*O*-allyl ether by treatment with dibutyltin oxide[33] and then allyl bromide. Standard benzylation with benzyl bromide and sodium hydride next afforded the protected thioglycoside **37** in 74% yield over two steps. Treatment with DIBAL-H in dichloromethane opened the 4,6-*O*-benzylidene acetal regioselectively to primary alcohol **38** exclusively in 89% yield (Scheme 8). Temperature plays an important role in determining the regiochemistry of this reaction, as does the nature of the 3-*O*-protecting group. In the case of 3-*O*-allyl-protected thiomannoside **37**, when the reaction was conducted at −78 °C, a 3:1 mixture of the regioisomers was obtained favoring the undesired secondary alcohol. Fortunately, at 0 °C, the primary alcohol was obtained exclusively. It was also necessary to add the solution of DIBAL-H slowly to avoid any removal of the acid-labile *p*-methoxybenzyl group. Conveniently, in the case of the 3-*O*-benzyl-protected thiomannoside **1**, no other regioisomer was obtained at −78 °C. Moreover, the *p*-methoxybenzyl group is less susceptible to removal at this temperature.

A number of one-carbon nucleophiles were surveyed with the aldehyde, obtained from **38** by Swern oxidation, in an attempt to shorten the synthesis of the heptose unit. These included organometallic reagents from benzyloxymethyl chloride,[6d,34] (benzyloxymethyl)tributylstannane,[35] and benzyl-

(33) (a) David, S.; Hanessian, S. *Tetrahedron* **1985**, *41*, 643. (b) David, S. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Dekker: New York, 1997; p 69.

(34) (a) Zamojski, A. *Polish J. Chem.* **2003**, *71*, 257. (b) Oscarson, S. *Top. Curr. Chem.* **1997**, *186*, 171. (c) Blomberg, C.; Hartog, F. A *Synthesis* **1977**, 18.

Table 2. Deprotection of the Disaccharides

Compound	Partial deprotection (% yield)[a]	Final product (% yield)[b]
23	 **30** (79)	 **31** (94)
24β	 **32** (75)	 **33** (95)
25	 **34** (72)	 **35** (92)

[a] Treatment with TBAF in THF followed by CSA in methanol. [b] Hydrogenolysis over Pd/C in methanol.

Scheme 8. Preparation of Heptoses **39** and **40**

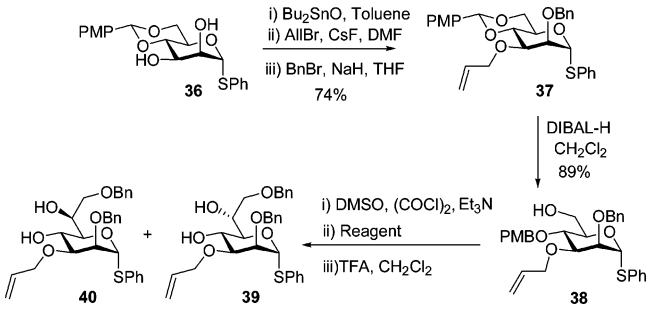
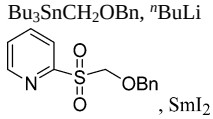


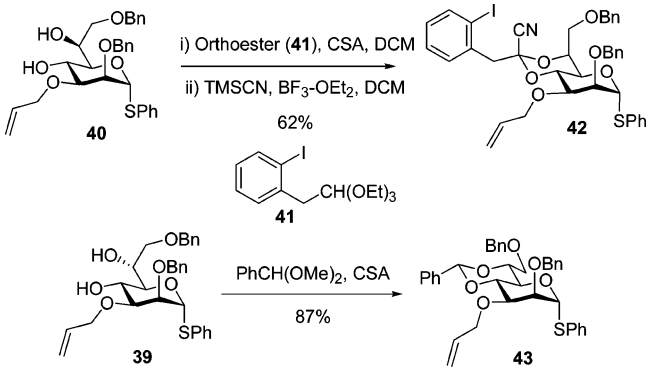
Table 3. Chain Elongation with Various One-Carbon Nucleophiles

reagent	% yield	**39** (D,D):**40** (L,D)
$BnOCH_2Cl$, Mg, $HgBr_2$	44	1:1.15
Bu_3SnCH_2OBn, ^{n}BuLi	41	1:1.15
 , SmI_2	38	1:1

oxymethyl 2-pyridyl sulfone.[36] The results of these reactions[37] are presented in Table 3.

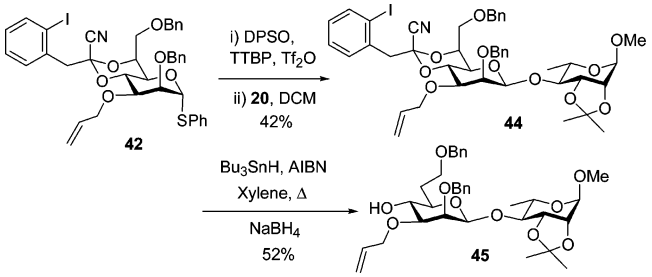
Treatment of the L,D-diol **40** with ortho ester **41**[41] in the presence of catalytic CSA followed by BF_3-OEt_2-promoted cyanation furnished the thioglycoside donor **42** in 62% yield over two steps. Similarly, the D,D-isomer **39** was treated with benzylidene dimethyl acetal and catalytic CSA to give D-*glycero*-D-*manno*-heptothioglycoside **43** in 87% yield (Scheme 9). The equatorial orientations of the hydroxymethyl group and benzylidene group in **43** were confirmed by NOE correlations. On the basis of stereoelectronic considerations[42] and previous X-ray crystollagraphic analysis,[16] it is inferred that the nitrile group in **42** adopts the axial orientation. Donor **42** was then glycosylated with **20** by prior activation with Ph_2SO/Tf_2O combination:[43] a stronger activator than BSP/Tf_2O required due to the strong electron-withdrawing, disarming nature of the nitrile group.[16] For the same reason, a higher temperature (−20 °C) is also required for complete activation. Under these conditions, disaccharide **44** was obtained with complete β-selectivity, albeit in moderate yield (42%) (Scheme 10). In the NMR spectrum of the reaction mixture, no other glycosylated product was observed. However, a complex mixture of compounds, in which both terminal and internal olefinic signals derived from the allyl group were clearly invisible, made up the mass balance, indicating incompatibility of the allyl group

Scheme 9. Preparation of *glycero-manno*-Heptoside Donors **42** and **43**



(35) (a) Fernández-Megía, E.; Ley, S. V. *Synlett* **2000**, *4*, 455. (b) Still, W. C. *J. Am. Chem. Soc.* **1978**, *100*, 1481.
(36) Skrydstrup, T.; Jespersen, T.; Beau, J.-M.; Bols, M. *Chem Commun.* **1996**, 515. The SmI_2 was freshly prepared prior to the reaction following Imamoto's protocol. Imamoto, T.; Ono, M. *Chem. Lett.* **1987**, *16*, 501.
(37) The reaction yields are presented for a three-step protocol involving Swern oxidation, chain elongation, and removal of the *p*-methoxybenzyl group with TFA.[38] Considering the aldehyde to be a poor electrophile,[39] anhydrous $CeCl_3$[40] was used in the case of Grignard reaction, but no significant improvement was observed.
(38) The diastereomeric mixture was separated only after removal of the *p*-methoxybenzyl group. Yan, L.; Kahne, D. *Synlett* **1995**, 523.
(39) Achkar, J.; Sanchez-Larraza, I.; Johnson, C. A.; Wei, A. *J. Org. Chem.* **2004**, *70*, 214.
(40) (a) Dimitrov, V.; Kostava, K.; Genov, M. *Tetrahedron Lett.* **1996**, *37*, 6787. (b) Evans, W. J.; Feldman, J. D.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 4581.
(41) The ortho ester **41** was prepared from 2-iodophenyl acetonitrile in 51% yield[16] and used without further purification.
(42) (a) Eliel, E.; Nadar, F. W. *J. Am. Chem. Soc.* **1970**, *92*, 584. (b) Eliel, E.; Nadar, F. W. *J. Am. Chem. Soc.* **1970**, *92*, 3050. (c) Rychnovsky, S. D.; Kim, J. *Tetrahedron Lett.* **1991**, *32*, 7223. (d) Wardrop, D. J.; Velter, A. I.; Forslund, R. E. *Org. Lett.* **2001**, *3*, 2261. (e) Wardrop, D. J.; Forslund, R. E.; Landrie, C. L.; Velter, A. I.; Wink, D.; Surve, B. *Tetrahedron: Asymmetry* **2003**, *14*, 929.
(43) (a) Codée, J. D. C.; Litjens, R. E. J. N.; den Heeten, R.; Overkleeft, H. S.; van Boom, J. H.; van der Marel, G. A. *Org. Lett.* **2003**, *5*, 1519. (b) van den Bos, L. J.; Codée, J. D. C.; van Boom, J. H.; Overkleeft, H. S.; van der Marel, G. A. *Org. Biomol. Chem.* **2003**, *1*, 4160.

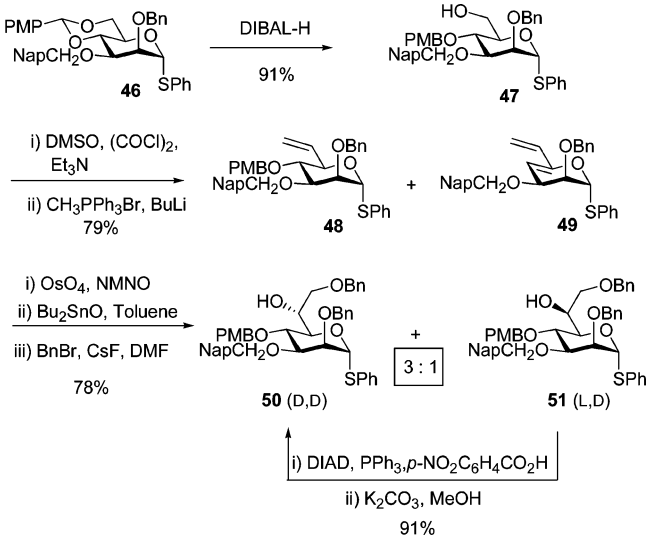
Scheme 10. Preparation of the Disaccharide **45**



with the glycosylation conditions applied.[44] Dropwise addition of tributyltin hydride and AIBN to a solution of the disaccharide **44** in xylene at reflux and subsequent removal of the tin residues with $NaBH_4$[46] afforded the 6-deoxy product **45** exclusively in 52% yield (Scheme 10). In the ^1H NMR spectrum of the reaction mixture, there was no indication of the formation of the regioisomeric 4-deoxy product. The hydrolysis of the 4-*O*-(2-cyanophenylacetyl) group, generated after the radical fragmentation, was the result of the $NaBH_4$ work up.

Synthesis of the Tetrasaccharide. On the basis of the above studies, a final strategy was evolved employing a benzyl-type ether protecting group on O3 of the eventual 6-deoxy heptoside donor, with all acetal protecting groups being introduced from the more favorable D,D-series. Moreover, the original Wittig olefination/osmoylation sequence for homologation was considered more practical than any of the organometallic methods investigated. Thus, the thioglycoside **46**, obtained by treatment of diol **36** with dibutyltin oxide and 2-naphthylmethyl bromide[47] followed by benzyl bromide and sodium hydride, was subjected to the sequence of reactions shown in Scheme 11 to give diols **50** and **51**. The stereochemistry at the C6 position of the minor L-*glycero*-D-*manno*-heptothioglycoside **51** was inverted by the Mitsunobu protocol (Scheme 11).

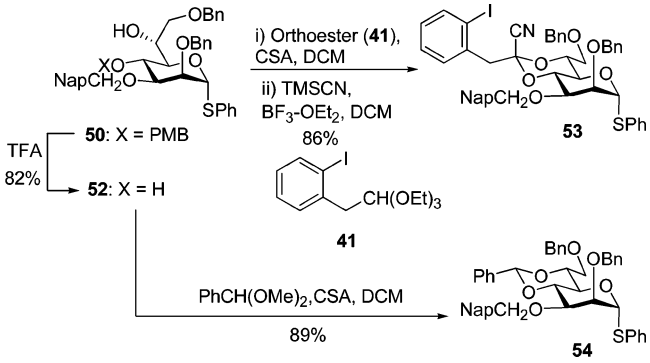
Scheme 11. Preparation of Heptoses **50** and **51**



It is noteworthy that **50** is protected by means of three benzyl-type ethers primed for selective removal. The sequence began with the removal of the *p*-methoxybenzyl group with TFA,[38]

(44) This is somewhat unexpected as allyl ethers had previously been shown to be compatible with the sulfoxide glycosylation.[8b] However, it should be noted that Mukaiyama has applied the Ph_2SO/Tf_2O combination to the functionalization of olefins.[45]

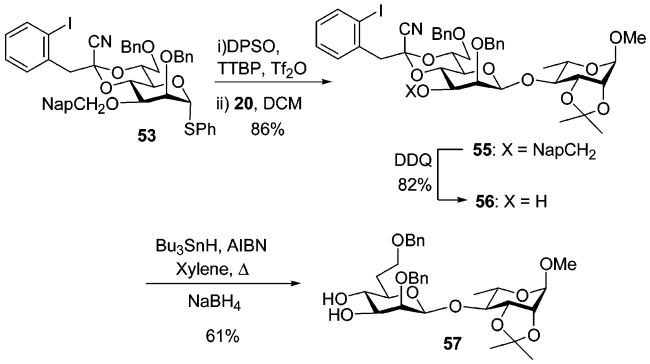
Scheme 12. Preparation of Donors **53** and **54**



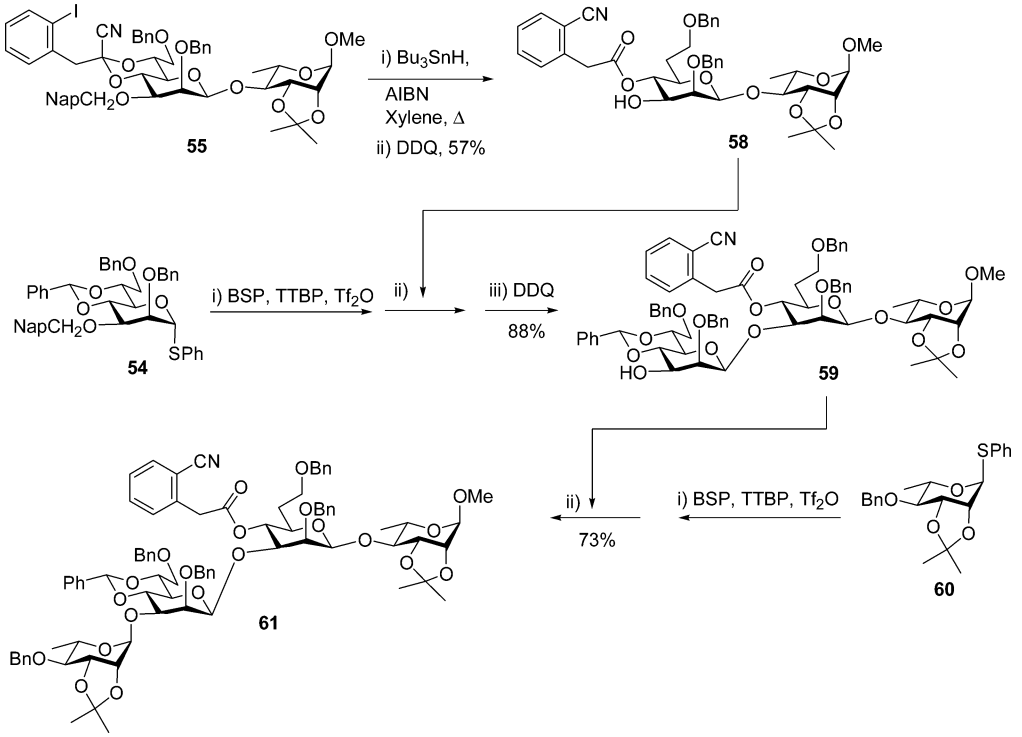
which furnished the diol **52** in 82% yield (Scheme 12). Treatment of thioglycoside **52** with ortho ester **41** in the presence of catalytic CSA followed by BF_3-OEt_2-promoted cyanation gave the thioglycoside donor **53**, precursor to the deoxyheptoside unit, in 86% yield. Similarly, D-*glycero*-D-*manno*-heptothioglycoside **54** was obtained by reacting **52** with benzaldehyde dimethyl acetal and catalytic CSA in 89% yield (Scheme 12). NOE correlations were used to verify the equatorial orientations of the benzyloxymethyl group and benzylidene group in **54** and, by implication, **53**.

The donor **53** was then glycosylated with methyl rhamnoside **20**, following preactivation with the Ph_2SO/Tf_2O combination in the presence of TTBP in dichloromethane at −20 °C, to give an 86% yield of the disaccharide **55** with complete β-selectivity (Scheme 13). Exposure of **55** to DDQ removed the 2-naphthylmethyl ether,[47c] providing alcohol **56** in 82% yield. Dropwise addition of tributyltin hydride and AIBN to a solution of the disaccharide **56** in xylene at reflux and subsequent removal of the tin residues with $NaBH_4$ afforded the 6-deoxy product **57** exclusively in 61% yield (Scheme 13). The NMR spectrum of the reaction mixture again showed no indication of the formation of the regioisomeric 4-deoxy product. Unfortunately, the $NaBH_4$ treatment hydrolyzed the 2-cyanophenyl acetyl ester generated on radical fragmentation. To avoid this problem, a solution of **55** in xylene at reflux was treated with tributyltin hydride and AIBN to afford the 6-deoxy-*manno*-heptopyranoside **58** as a single regioisomer. The reaction mixture was subsequently treated with DDQ to remove the 2-naphthylmethyl group. This oxidative treatment also facilitated chromatographic purification and eliminated the need for the $NaBH_4$ treatment. The disaccharide acceptor alcohol **58** was obtained in 57% yield over the two steps (Scheme 14).

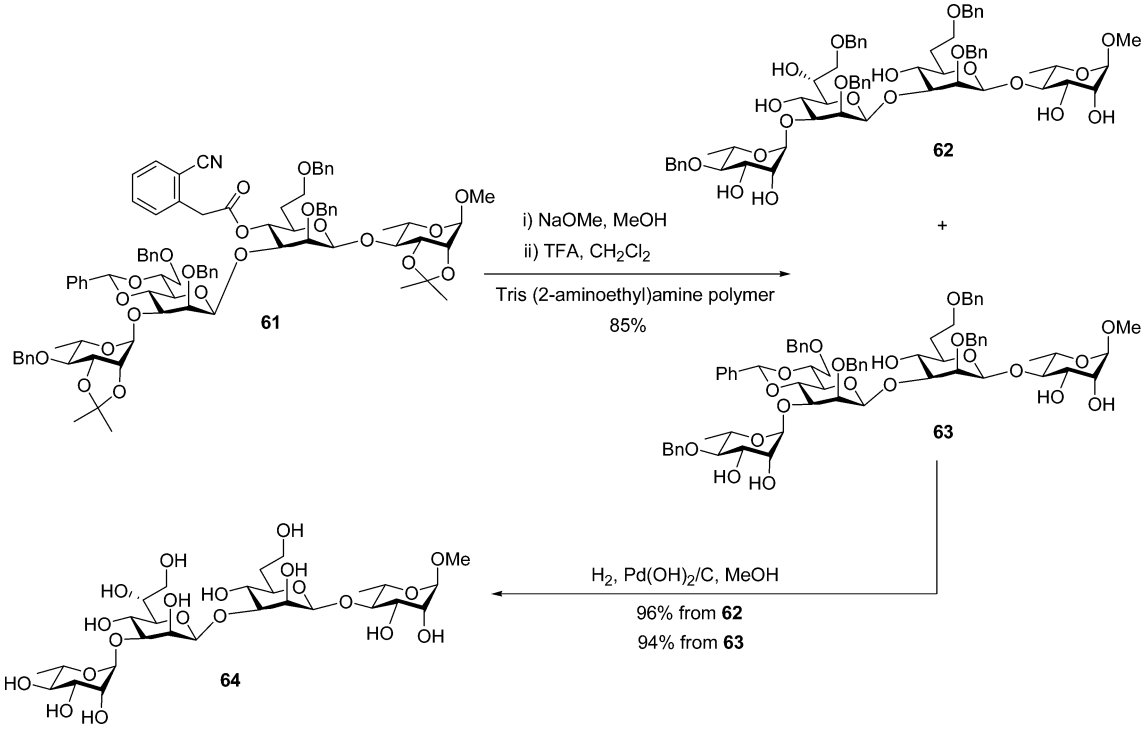
Scheme 13. Preparation of the Disaccharide **57**



Scheme 14. Preparation of the Tetrasaccharide



Scheme 15. Completion of the Synthesis



Synthesis of the desired trisaccharide was performed by activating the D-*glycero*-D-*manno*-heptothioglycoside **54** with BSP and Tf_2O in the presence of TTBP in dichloromethane at −60 °C, followed by addition of **58** in dichloromethane. Subsequent removal of the 2-naphthylmethyl ether with DDQ furnished the trisaccharide alcohol **59** in 88% yield (Scheme 14). The stage was now set for the final glycosylation reaction to furnish the tetrasaccharide. Therefore, the rhamnosyl donor **60**[48] was activated under standard BSP/Tf_2O conditions and

(45) (a) Matsuo, J−i.; Yamanaka, H.; Kawana, A.; Mukaiyama, T. *Chem. Lett.* **2003**, *32*, 392. (b) Yamanaka, H.; Mukaiyama, T. *Chem. Lett.* **2003**, *32*, 1192. (c) Yamanaka, H.; Matsuo, J−i.; Kawana, A.; Mukaiyama, T. *Chem. Lett.* **2003**, *32*, 626.

(46) Crich, D.; Sun, S. *J. Org. Chem.* **1996**, *61*, 7200.

(47) (a) Gaunt, M. J.; Yu, J.; Spencer, J. B. *J. Org. Chem.* **1998**, *63*, 4172. (b) Wright, J. A.; Yu, J.; Spencer, J. B. *Tetrahedron Lett.* **2001**, *42*, 4033. (c) Xia, J.; Abbas, S. A.; Locke, R. D.; Piskorz, C. F.; Alderfer, J. L. *Tetrahedron Lett.* **2000**, *41*, 169.

(48) Zegelaar-Jaarsveld, K.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron* **1992**, *48*, 10133.

Table 4. Comparison of Anomeric ^{1}H and ^{13}C NMR Chemical Shifts (ppm)

	^{1}H NMR		^{13}C NMR	
residues	isolated[a]	synthetic	isolated[a]	synthetic ($^1J_{CH}$)
−4)-α-L-rha*p*-(1-(reducing end)	4.85 (4.83)	4.56	102.1 (101.9)	101.3 (163.0 Hz)
−3)−6d-*β*-D-*manno*-Hep*p*-(1-[b]	5.02 (5.02)	4.70	100.5 (100.3)	100.8 (157.6 Hz)
−3)-D-*glycero*-*β*- D-*manno*-Hep*p*-(1-	4.76 (4.74)	4.77	97.9 (97.8)	96.8 (157.4 Hz)
−4)-α-L-rha*p*-(1-(nonreducing end)	4.95 (4.91)	4.92	97.3 (96.7)	96.1 (166.1 Hz)

[a] The data collected from ref 7a, whereas the data in parentheses have taken from ref 7b. [b] The O2 is acylated in the polysaccharide.

Table 5. Comparison of ^{1}H and ^{13}C NMR Chemical Shifts (ppm) at the 6-Position

	^{1}H NMR				^{13}C NMR	
	H6		H6′		C6	
residues	isolated[a]	synthetic	isolated[a]	synthetic	isolated[a]	synthetic
−4)-α-L-rha*p*-(1-(reducing end)	1.26 (1.24)	1.25	−	−	17.7 (17.6)	16.6
−3)−6d-*β*-D-*manno*-Hep*p*-(1-	1.75 (1.75)	1.68−1.75	2.17 (2.18)	2.14−2.21	34.4 (34.4)	34.5
−4)-α-L-rha*p*-(1-(nonreducing end)	1.29 (1.32)	1.33	−	−	17.9 (18.1)	17.0

[a] The data collected from ref 7a, whereas the data in parentheses have taken from ref 7b.

allowed to react with **59** to afford the tetrasaccharide as a single α-stereoisomer in 73% yield, following the established pattern.[49] Saponification of the (2-cyanophenyl)acetyl ester with sodium methoxide in methanol and subsequent treatment with TFA[49] in dichloromethane followed by quenching the excess TFA with tris(2-aminoethyl) amine[50] afforded a mixture of **62** and **63** in 85% yield over two steps (Scheme 15).[51] These were subjected individually to hydrogenolysis over Pearlman's catalyst giving the tetrasaccharide **64** in 96% and 94% yields, respectively. Representative ^{1}H and ^{13}C NMR chemical shift data for this unit contained within the LPS from *Plesimonas shigelloides*[7] are presented in Tables 4 and 5. The differences between the two sets of data most evident toward the reducing end, probably arise from the synthetic material being a methyl glycoside rather than the much more hindered glycosidic bond in the natural isolate.

(49) Crich, D.; Li, H. *J. Org. Chem.* **2002**, *67*, 4640.
(50) Booth, R. J.; Hodges, J. C. *J. Am. Chem. Soc.* **1997**, *119*, 4882.
(51) Extended reaction time led to the onset of decomposition.

In summary, a method for the stereocontrolled synthesis of both *β*-D-*glycero*-D-*manno*- and *β*-L-*glycero*-D-*manno*-heptopyranosides has been developed, making possible a total synthesis of the core tetrasaccharide unit present in the hexasaccharide repeating unit of the LPS *Plesimonas shigelloides*. The 1-cyano-2-(2-iodophenyl)ethylidene group was successfully employed as a 4,6-*O*-benzylidene surrogate to construct the 6-deoxy-*β*-Hep*p* unit by a reductive radical fragmentation method.

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Supporting Information Available: Full experimental and characterization details for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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