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Studies towards the total synthesis of repeating unit of *O*-sulfated polysaccharide from marine bacterium *Cobetia pacifica* KMM 3878

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Herein we report assembly of the appropriately protected trisaccharide repeating unit of *Cobetia pacifica* KMM 3878 *O*-sulfated polysaccharide. Our synthesis involves 3,4-*O*-pyruvilated galactose as the key building block which acts as a donor as well as acceptor in the construction of trisaccharide. We obtained the *R* isomer as a major stereoisomer in the pyruvilation reaction. The glycosylations proceeded with high stereo and regioselectivity.

Keywords: 3,4-*O*-Pyruvilated galactose, regioselective glycosylation, Gram-negative bacteria.

Introduction

In 2014, Kokulin and co-workers isolated a biologically active *O*-specific polysaccharide (OPS) from the lipopolysaccharide of *Cobetia pacifica* KMM 3878 found on the sandy sediment of the sea of Japan. The structure of OPS was studied by chemical methods along with 1D and 2D NMR spectroscopy[1]. The *O*-specific polysaccharide contains trisaccharide repeating unit →4)-β-D-Gal2,3R-(1→6)-β-D-Gal3,4(S-Pyr)-(1→6)-β-D-Gal-(1→, where R is -SO_3H. The repeating unit of OPS from *C. pacifica* KMM 3878 presented the disulfated trisaccharide, which is uncommon for Gram-negative bacteria *O*-antigens. A distinctive feature of the *O*-specific polysaccharide KMM 3878 is the presence of 2,3-*O*-disulfate-D-galactose. This is the first monosaccharide with two sulfate groups found in Gram-negative bacteria OPS. Another uncommon sugar is 3,4-*O*-[(*S*)-1-carboxyethylidene]-D-galactose (pyruvilated galactose) which is frequent component of bacterial OPS[2–6].

Retrosynthetic strategy

Our retrosynthetic strategy for the synthesis of sulfated *O*-specific polysaccharide **1** is outlined in Scheme 1. Functional group interconversion and Global deprotection of the appropriately protected trisaccharide **2** would afford the target molecule **1**. The protected trisaccharide **2** can be obtained by coupling of disaccharide 6-OH acceptor corresponding to **4** with thiogalactosyl donor **3** which can be obtained from easily available D-galactose. Coupling of **6** with acceptor **5** is expected to produce disaccharide **4** with desired β-selectivity due to neighbouring group participation of C2 OBz group. Both compounds **5** and **6** can be obtained from D-galactose.

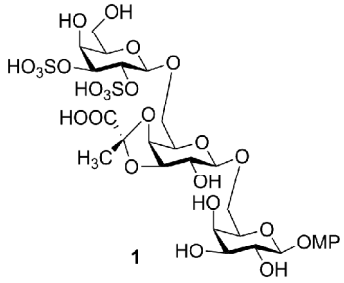

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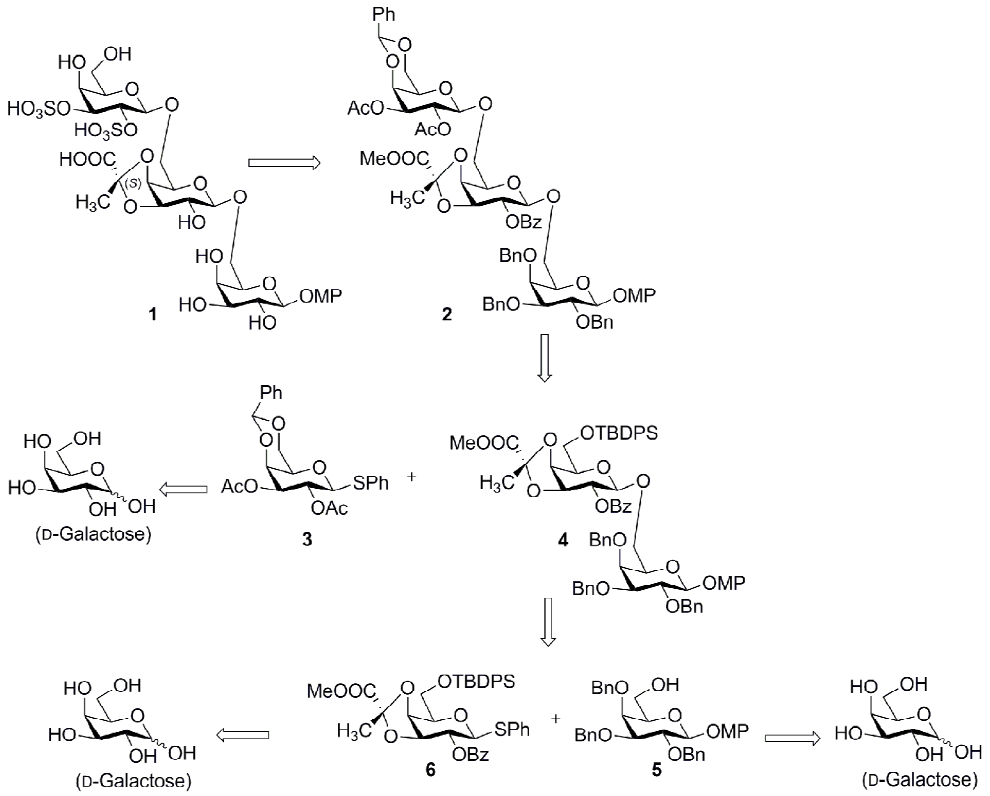
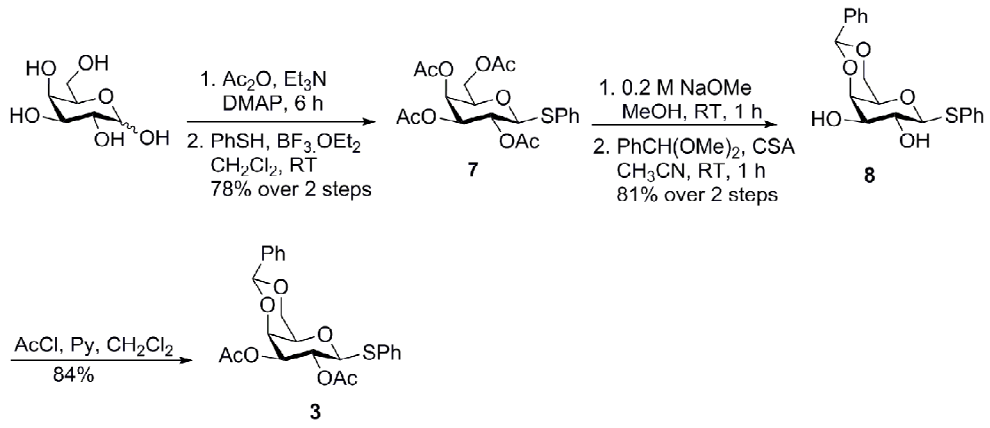
Fig. 1. Structure of trisaccharide **1**.

As shown in Scheme 1, for the synthesis of trisaccharide molecule **1**, all monosaccharide building blocks synthesis was started from commercially available D-galactose. In first step, per-*O*-acetylation of D-galactose by using acetic anhydride, triethylamine and cat. DMAP afforded peracetylated compound in a quantitative yield. Then the nucleophilic displacement of anomeric acetate group by thiophenol using Lewis acid $BF_3{\cdot}OEt_2$ gave desired β thiogalactoside **7** in 78% yield over two steps (Scheme 2). Compound **7** upon treatment with NaOMe in MeOH afforded tetraol compound quan-



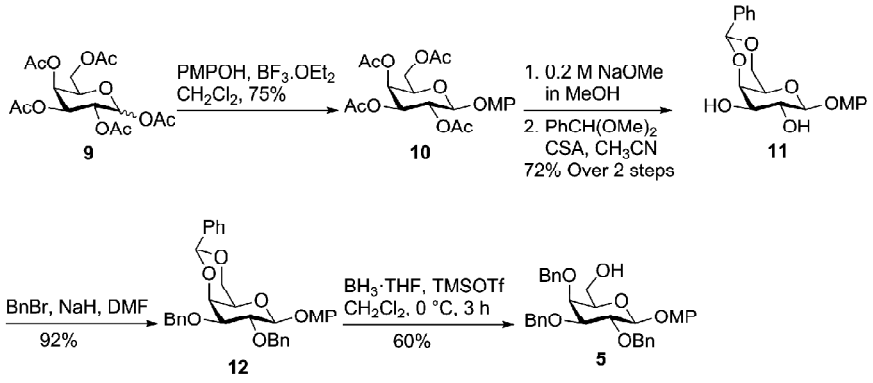
Scheme 1. Retro synthetic analysis.



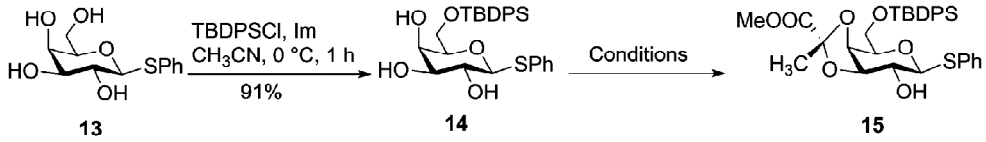
Scheme 2. Synthesis of thiogalactoside building blocks.

titatively which was subjected to benzylidene protection using benzaldehyde dimethyl acetal, and camphor sulfonic acid (CSA) in CH_3CN to get compound **8** in good yield (81% over two steps). Subsequently, acetylation of C2 and C3 hydroxyl groups was carried out by using acetyl chloride, and pyridine in CH_2Cl_2 to get the desired building block **3** in 84% yield.

After successful synthesis of building block **3**, we went ahead for the synthesis of building block **5** (Scheme 3). Nucleophilic displacement of anomeric acetate group of compound **9** using $BF_3{\cdot}OEt_2$ and *p*-methoxyphenol gave the desired β-product **10** in 75% yield over two steps. The acetate groups in **10** were hydrolyzed under Zemplén conditions



Scheme 3. Synthesis of acceptor **5**.



Scheme 4. Attempted synthesis of compound **15**.

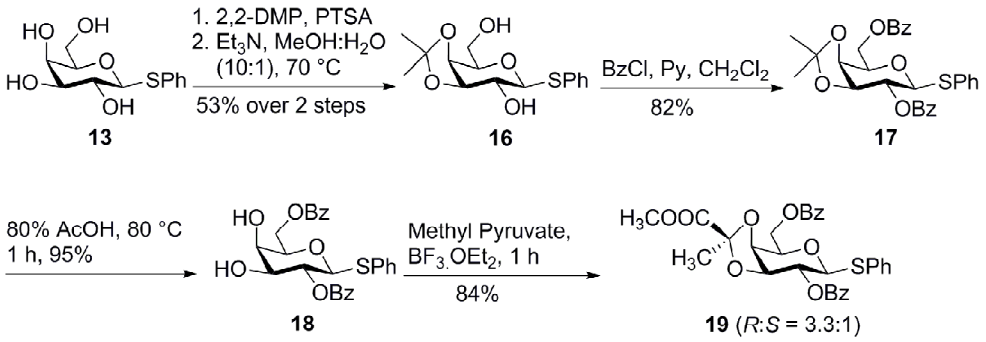
(NaOMe, MeOH) to get tetraol compound. Then the free C4 and C6 hydroxyl groups of tetrol compound were protected as benzylidene acetal by using benzaldehyde dimethyl acetal and camphor sulphonic acid (CSA) in acetonitrile solvent to give 4,6-benzylidene protected diol **11** in 72% yield over two steps. The free hydroxyl groups were benzylated by using BnBr and NaH to afford compound **12** in 92% yield. BH_3·THF, TMSOTf mediated reductive benzylidene ring opening[2] of compound **12** gave 6-OH acceptor **5** with 60% yield[7].

Synthesis of pyruvilated galactose compound **6** was carried out from tetraol compound **13**. First,treatment of **13** with TBDPSCl, Im in CH_3CN gave triol compound **14** in 91% yield. We then attempted methyl pyruvate protection on C3 and C4 hydroxyl groups with desired *S* configuration under several different conditions (see Table 1).

Since we were not successful in incorporating the methyl pyruvilated protection at C3 and C4 position in the presence of the bulky TBDPS group, we attempted to synthesize another thiogalactoside donor **19** devoid of TBDPS. Compound **13** was reacted with 2,2-dimethoxy propane, dry PTSA for 3 h at room temperature. After that the reaction mixture was dissolved in MeOH:H_2O (10:1) and refluxed at 70°C for 5 h to get 3,4-acetonide protected compound **16**. Subsequently benzoylation of C2 and C6 hydroxyl groups was carried out with benzoyl chloride in presence of pyridine to get compound **17** in 82% yield. Acetonide group was removed in 80% acetic acid solution at 80°C to get C3 and C4 free hydroxyl compound **18** in good yield. Treatment of compound **18** with methyl pyruvate in the presence of strong Lewis acid BF_3·OEt_2 gave diastereomeric mixture of compound **19** in 84% yield with undesired *R* isomer in major amount (*R*:*S* =

Table 1. Conditions for methyl pyruvate protection on compound **14**

No.	Reaction conditions	Time	Results
1.	$CH_3COCOOMe$ (1.1 equiv.), TMSOTf (1.1 equiv.), CH_3CN (1 mL)	45 min	Tetraol **9** was obtained
2.	$CH_3COCOOMe$ (24 equiv.), TMSOTf (2 equiv.)	1 h	Tetraol **9** was obtained
3.	$CH_3COCOOMe$ (24 equiv.), BF_3·OEt_2 (2 equiv.)	1 h	Tetraol **9** was obtained
4.	$CH_3COCOOMe$ (4 equiv.), TMSOTf (0.4 equiv.), CH_3CN (1 mL), MS 3 Å	24 h	Compound **14** was recovered
5.	$CH_3COCOOMe$ (24 equiv.), BF_3·OEt_2 (2 equiv.), MS 3 Å	24 h	Compound **14** was recovered



Scheme 5. Synthesis of compound **19**.

3.3:1)[5]. We were able to purify the *R* isomer whereas the minor *S* isomer always accompanied by some *R* isomer. Configuration of the major *R* isomer was confirmed by NOESY experiment which shows correlation between H-4 and -CH_3 group[1]. The *S* isomer was expected to show a correlation between CH_3 group and H-2 which was absent in our spectrum.

Stereo selectivity on compound **19** was confirmed by NMR spectroscopic method. NOESY experiments shows correlation of -CH_3 proton with H-4 proton but not with H-2 proton, which confirms the undesired selectivity (*R* isomer) in compound **19**. The ^{1}H NMR and ^{13}C data of our compound matched well with its reported data[5].

Though we were not able to get the desired selectivity in pyruvilation, we proceeded with the *R* isomer of **19** to investigate its reactivity towards glycan assembly. First, pyruvilated thiogalactoside donor **19** was converted to its corresponding bromide using Br_2 in CH_2Cl_2 at 0°C. So, the formed bromide was treated with acceptor **6** in the presence of AgOTf and sym. collidine as the promoter at –30°C to afford disaccharide **20** in 61% yield in β selectivity. It should be noted that use of the more common promoter system NIS/TMSOTf at –30°C afforded **20** in 27% yield only. Debenzoylation of compound **20** using NaOMe in MeOH afforded compound **21** in

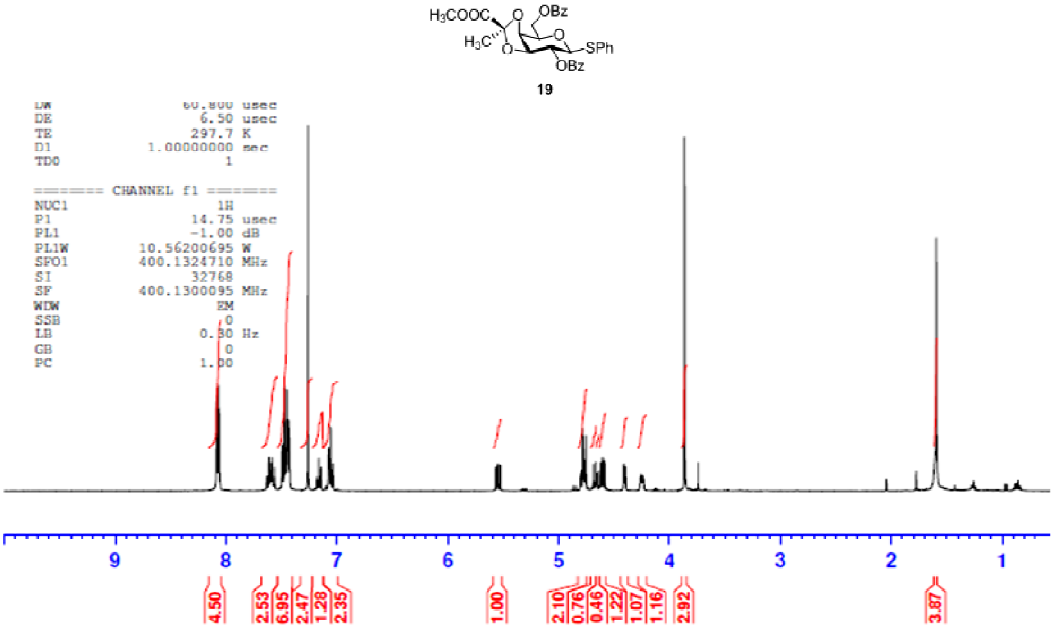


Fig. 2. ^{1}H NMR of compound **19**.

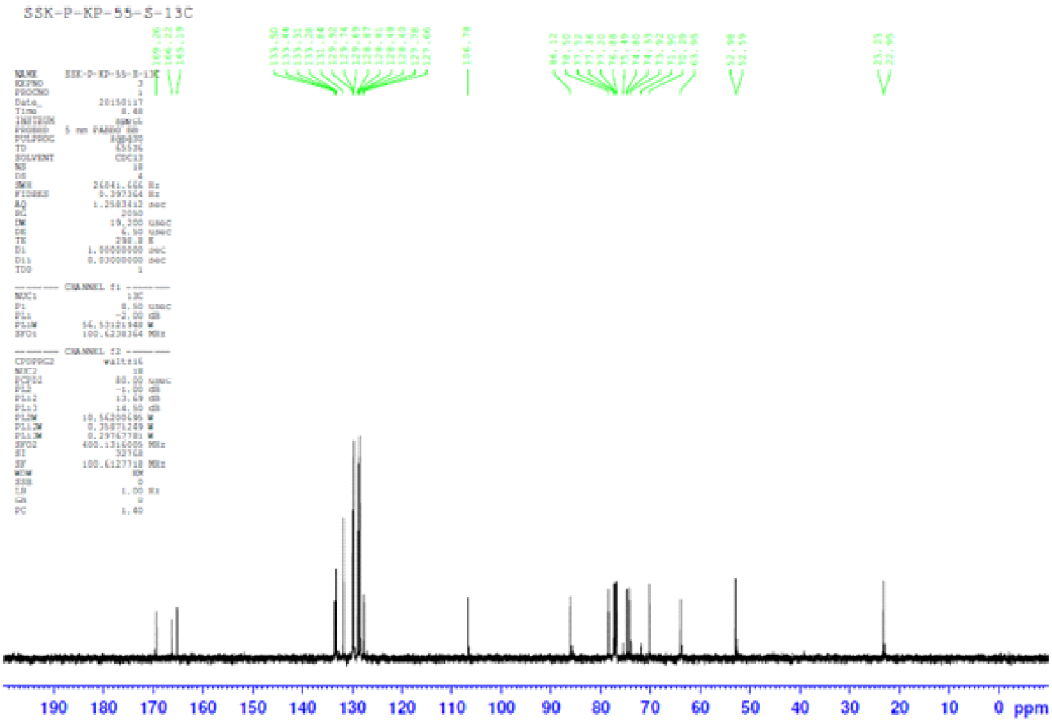


Fig. 3. ^{13}C NMR of compound **19**.

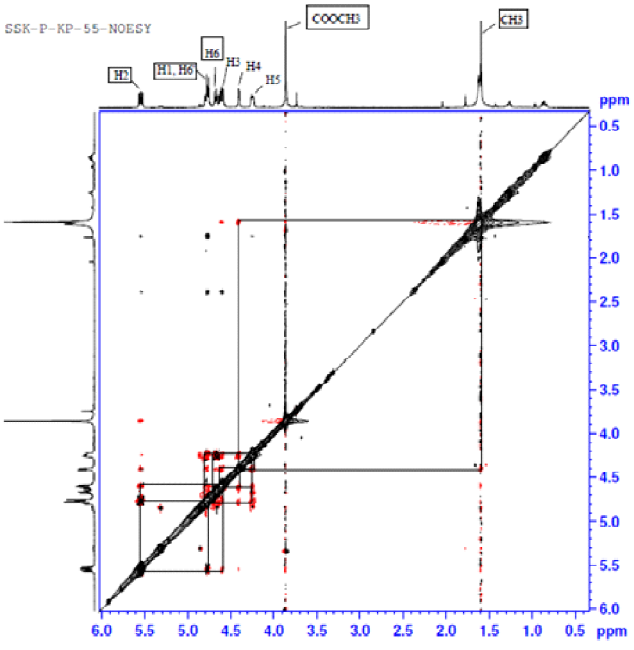


Fig. 4. NOESY of compound **19**.

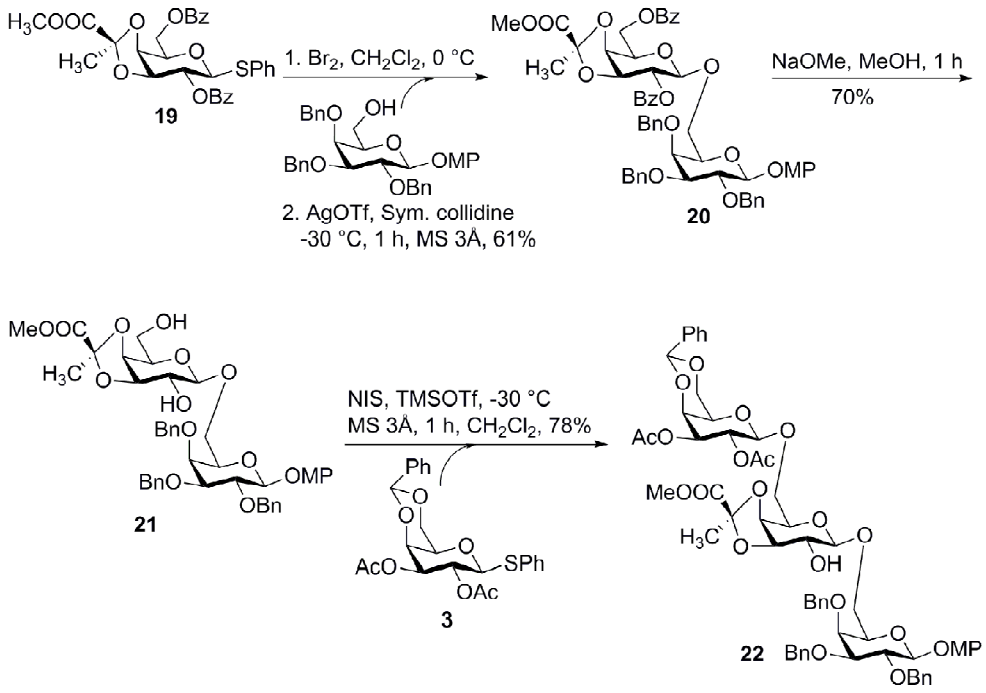
70% yield. Disaccharide diol compound **21** was coupled with thiogalactoside donor **3** under NIS/TMSOTf as the promoter at –30°C to furnish β-linked trisaccharide **22** in 78% yield in a highly regio and steroselective manner.

Conclusion

We successfully completed the assembly of a putative trisaccharide repeating unit of *Cobetia pacifica* KMM 3878 in an efficicent manner. Since we were no able to synthesize the pyruvilated trisaccharide compound **22** with the requisite stereoselectivity, further deprotection and sulfation steps were not carried out. Further work to reverse the selectivity of pyruvilation using chiral Lewis acids is under progress.

Experimental

General information: All reactions were conducted under the dry nitrogen atmosphere. Solvents (CH_2Cl_2>99%, THF 99.5%, acetonitrile 99.8%, DMF 99.5%) were purchased in capped bottles and dried under sodium or CaH_2. All other solvents and reagents were used without further purification. All glassware used was oven dried before use. TLC was performed on pre-coated aluminium plates of silica gel 60 F254 (0.25 mm, E. Merck). Developed TLC plates were visualized under a short-wave UV lamp and by heating plates that were dipped in ammonium molybdate/cerium(IV) sulfate



Scheme 6. Synthesis of trisaccharide.

solution. Silica gel column chromatography was performed using silica gel (100–200 mesh) and employed a solvent polarity correlated with TLC mobility. We have used 3 Angstrom powdered molecular sieves in our study. The powdered MS were weighed in a dried pear shaped flask and activated by periodic heating of flask by using flame over 15 min period.

NMR experiments were conducted on 500 and 400 MHz instrument using $CDCl_3$ (D, 99.8%) or D_2O (D, 99.9%) as solvents. Chemical shifts are relative to the deuterated solvent peaks and are in parts per million (ppm). ^{1}H-^{1}H COSY was used to confirm proton assignments. Mass spectra were acquired in the ESI mode.

*Phenyl-2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranoside (**7**):*

Et_3N (310 mL, 222.20 mmol) was added to the *vacuum* dried D-galactose (20 g, 110.01 mmol), after 5 min Ac_2O (52.4 mL, 555.05 mmol) and DMAP (1.35 g, 11.10 mmol) were added at 0°C. After consumption of starting material (monitored in TLC), Et_3N was evaporated *in vaccuo,* crude product was dissolved in ethyl acetate (250 mL) and it was washed with $NaHCO_3$ solution and water. Separated organic layer was dried over Na_2SO_4 and concentrated *in vaccuo* to obtain peracetylated compound as brown viscous liquid (quantitative yield).

Crude compound (30.5 g, 78.14 mmol) was dissolved in CH_2Cl_2 (250 mL), and PhSH (16 mL, 156.30 mmol) was added at 0°C. After 5 min, $BF_3{\cdot}OEt_2$ (20 mL, 156.30 mmol) was added in dropwise manner and reaction was stirred at room temperature for 12 h. After completion of the reaction, organic layer was dissolved in CH_2Cl_2 (500 mL) and washed with aq. $NaHCO_3$ solution. Separated organic layer was dried over anhydrous Na_2SO_4, concentrated, and purified by column chromatography silica gel (15% ethyl acetate/pet ether) to give compound **8** as a viscous liquid (27.8 g, 78%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53–7.47 (m, 3H, ArH), 7.32–7.21 (m, 2H, ArH), 5.67 (d, *J* 3.0 Hz, 1H, H-2), 5.28 (t, *J* 10.0 Hz, 1H, H-4), 5.06 (dd, *J* 10.0, 3.0 Hz, 1H, H-3), 4.91 (s, 1H, H-1), 4.29 (dd, *J* 12.0, 6.4 Hz, 1H, H-6a), 4.17 (dd, *J* 12.0, 2.4 Hz, 1H, H-6b), 3.73–3.68 (m, 1H, H-5), 2.21 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.2, 170.0, 169.6, 133.2, 131.9, 129.1, 128.1, 85.5, 76.4, 71.8, 70.6, 65.7, 62.8, 20.8, 20.7, 20.6, 20.5; HR-ESI-MS (*m/z*): $[M+Na]^+$ Calcd. for $C_{20}H_{24}NaO_9S$ 463.1039, Found: 463.1037.

*Phenyl-4,6-O-benzilidene-1-thio-β-D-galactopyranoside (**8**):*

NaOMe (1.62 g, 30 mmol) was added to a stirred solution of compound **7** (12.5 g, 32.90 mmol) in MeOH (150 mL). Then the mixture was stirred for 1 h. After completion of reaction, amberlite (strong acid, 13 gm) was added. Then compound was filtered, washed with MeOH, concentrated and dried under high vacuum to give tetraol compound quantitatively as brown viscous liquid.

To the solution of crude tetraol compound (4.4 g, 16.16 mmol) in CH_3CN (35 mL), camphor sulphonic acid (1.87 g, 8.08 mmol), $PhCH(OMe)_2$ (3 mL, 19.39 mmol) were added. After 1 h solvents were removed under reduced pressure. The obtained residue was purified by column chromatography silica gel (70% ethyl acetate/pet ether) to give compound **8** as white solid (4.7 g, 81%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66–7.65 (m, 2H, ArH), 7.646–7.40 (m, 2H, ArH), 7.402–7.34 (m, 3H, ArH), 7.29–7.255 (m, 3H, ArH), 5.46 (s, 1H, PhCH), 4.44 (d, *J* 9.08 Hz, 1H, β), 4.32 (dd, *J* 12.4, 1.44 Hz, 1H, H-2), 4.09 (dd, *J* 3.2, 0.8 Hz, 1H, H-5), 3.95 (dd, *J* 12.4, 1.6 Hz, 1H, H-3), 3.68–3.60 (m, 2H, H-6′, H-6″), 3.41 (d, *J* 0.96 Hz, 1H, H-4), 3.06 (bs, 2H, OH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 137.6, 133.6, 131.4, 129.2, 128.9, 128.24, 128.2, 126.6, 101.2, 85.2, 73.5, 73.2, 69.8, 69.1, 66.9; HR-ESI-MS (*m/z*): $[M+Na]^+$ Calcd. for $C_{19}H_{20}NaO_5S$ 383.4241, Found: 383.4238.

*Phenyl-2,3-O-acetyl-4,6-O-benzilidene-1-thio-β-D-galactopyranoside (**3**):*

AcCl (1.22 mL, 17.23 mmol) and pyridine (1.4 mL, 17.23 mmol) were added to a stirred solution of compound **8** (1.03 g, 2.87 mmol) in CH_2Cl_2 (10 mL) at 0°C. After 3 h reaction mixture was diluted with CH_2Cl_2 (25 mL), and the obtained organic layer was washed with water and brine solution, separated organic layer was dried over anhydrous Na_2SO_4, concentrated, and purified by column chromatography on silica gel (20% ethyl acetate/pet ether) to give compound **3** as white solid (1.07 g, 84%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61–7.59 (m, 2H, ArH), 7.39–7.35 (m, 5H, ArH), 7.31–7.23 (m, 3H, ArH), 5.45 (s, 1H, PhCH), 5.33 (t, *J* 9.8 Hz, 1H, H-2), 4.99 (dd, *J* 9.9, 3.3 Hz, 1H, H-3), 4.69 (d, *J* 9.8 Hz, 1H, β), 4.35–4.32 (m, 2H, H-6), 3.99 (d, *J* 14 Hz, 1H, H-5), 3.55 (s, 1H, H-4), 2.16 (s, 3H, $OCOCH_3$), 2.05 (s, 3H, $OCOCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.8,169.2, 137.6, 133.6, 131.4, 129.2, 128.9, 128.24, 128.2, 126.6, 101.2, 85.2, 73.5, 73.2, 69.8, 69.1, 66.9, 21.0; HR-ESI-MS (*m/z*): $[M+Na]^+$ Calcd. for $C_{23}H_{24}NaO_7S$ 467.49746, Found: 467.4964.

*4-Methoxyphenyl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (**10**):*

p-Methoxyphenol (1.91 g, 15.4 mmol) and $BF_3{\cdot}OEt_2$ (2 mL, 15.4 mmol) were added to a stirred solution of crude per acetate compound **9** (5.0 g, 12.8 mmol) in CH_2Cl_2 (30 mL) at 0°C. After 12 h reaction mixture was diluted with CH_2Cl_2 (100 mL), and the obtained organic layer was washed with aq. $NaHCO_3$ solution. Separated organic layer was dried over anhydrous Na_2SO_4, concentrated and purified by column chromatography over silica gel (20% ethyl acetate/pet ether) to give compound **10** as white solid (4.4 g, 75%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.94–6.91 (m, 2H, ArH), 6.79–6.77 (m, 2H, ArH), 5.44–5.39 (m, 2H, H-4, H-5), 5.06 (dd, *J* 10.4, 3.4 Hz, 1H, H-3), 4.89 (d, *J* 8 Hz, 1H, β), 4.22–4.17 (m, 1H, H-6a), 4.14–4.10 (m, 1H, H-6b), 3.98–4.00 (m, 1H, H-2), 3.73 (s, 3H, OCH_3), 2.15 (s, 3H, $OCOCH_3$), 2.05 (s, 3H, $OCOCH_3$), 2.02 (s, 3H, $OCOCH_3$), 1.97 (s, 3H, $OCOCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.4, 170.3, 170.2, 169.5, 155.9, 151.1, 118.7, 114.6, 101.0, 71.0, 70.9, 68.8, 67.01, 61.4, 55.7, 20.8, 20.7, 20.6; HR-ESI-MS (*m/z*): $[M+Na]^+$ Calcd. for $C_{21}H_{26}NaO_{11}$ 477.4245, Found: 477.4243.

*4-Methoxyphenyl-4,6-O-benzilidene-β-D-galactopyranoside (**11**):*

NaOMe (0.17 g, 3.2 mmol) was added to a stirred solution of compound **10** (1.5 g, 3.48 mmol) in MeOH (16 mL). After 1 h, to this reaction mixture amberlite (strong acid, 1.6 g) was added. Then solvent was filtered and evaporated to give tetraol as a white solid.

Camphor sulfonic acid (0.54 g, 0.5 mmol), $PhCH(OMe)_2$ (0.85 mL, 5.66 mmol) were added to stirred solution of tetraol (1.35 g, 4.71 mmol) in CH_3CN (11 mL). After 1.5 h solvents were removed under reduced pressure, obtained residue was purified by column chromatography over silica gel (40% ethyl acetate/pet ether) to give **11** as a white solid (1.27 g, 72%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53–7.51 (m, 2H, ArH), 7.38–7.36 (m, 3H, ArH), 7.07–7.05 (m, 2H, ArH), 6.83–6.81 (m, 2H, ArH), 5.57 (s, 1H, PhCH), 4.79 (d, *J* 7.7 Hz, 1H), 4.36 (d, *J* 12 Hz, 1H), 4.26 (s, 1H), 4.08 (d, *J* 11.5 Hz, 1H), 4.00 (d, *J* 9.4 Hz, 1H) (s, 4H, -OCH_3, 1H), 3.57 (s, 1H), 2.37 (bs, 2H, -OH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.8, 151.3, 137.61, 137.6, 137.4, 128.5, 126.6, 119.2, 114.7, 102.6, 101.7, 75.3, 72.8, 71.7, 69.2, 66.5, 55.8; HR-ESI-MS (*m/z*): $[M+Na]^+$

Calcd. for $C_{20}H_{22}NaO_7$ 397.3845, Found: 397.3849.

*4-Methoxyphenyl-4,6-O-benzilidene-2,3-di-O-benzyl-β-D-galactopyranoside (**12**):*

Compound **11** (0.35 g, 0.93 mmol) was dissolved in DMF (6 mL). Then NaH (0.06 g, 2.34 mmol) and BnBr (0.3 mL, 2.34 mmol) were added sequentially at 0°C. After complete conversion of starting material (confirmed by TLC) reaction mixture was diluted with ethyl acetate (20 mL), obtained organic layer was washed with water and brine solution in several times. The separated organic layer was dried over anhydrous Na_2SO_4, concentrated, and purified by column chromatography over silica gel (20% ethyl acetate/pet ether) to give compound **12** as a white solid (0.46 g, 89%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55–7.50 (m, 2H, ArH), 7.5–7.25 (m, 13H, ArH), 7.15–7.10 (m, 2H, ArH), 6.91–6.87 (m, 2H, ArH), 5.5 (s, 1H, PhCH), 5.11 (d, *J* 12 Hz, 1H, -CHPh), 4.89–4.85 (m, 2H), 4.78 (d, *J* 8 Hz, 2H), 4.33 (d, *J* 12.4 Hz, 1H), 4.16 (s, *J* 3.4 Hz, 1H), 4.12–4.10 (m, 3H), 3.77 (s, 3H, -OCH_3), 3.65 (dd, *J* 9.7, 3.6 Hz, 1H), 3.41 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.5, 151.8, 139.5, 138.6, 137.2, 129.2, 128.7, 128.8, 128.5, 128.4, 128.38, 128.36, 128.3, 128.04, 128.0, 127.9, 127.8, 126.7, 119.8, 119.5, 118.6, 118.3, 114.6, 114.0, 103.4, 101.6, 79.3, 78.3, 76.94, 75.6, 73.9,72.3, 69.3, 66.7, 55.6; HR-ESI-MS (*m/z*): $[M+Na]^+$ Calcd. for $C_{34}H_{34}NaO_7$ 577.62956, Found: 577.6293.

*4-Methoxyphenyl-2,3,4-tri-O-benzyl-β-D-galactopyranoside (**5**):*

To a stirred solution compound **12** (0.67 g, 1.21 mmol) in dry CH_2Cl_2 (6 mL), BH_3·THF (6 mL, 6.04 mmol) was added dropwise. After 5 min, TMSOTf (0.03 mL, 0.18 mmol) was added dropwise at 0°C and stirred for 3 h. After complete consumption of starting material (confirmed by TLC), MeOH was added to quench BH_3·THF. Then organic solvents were evaporated, obtained residue was purified by column chromatography over silica gel (40% ethyl acetate/pet ether) to give **5** as a white solid (0.4 g, 60%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.75–7.30 (m, 15H, ArH), 7.00 (d, *J* 4.9 Hz, 2H, ArH), 6.81(d, *J* 4.7 Hz, 2H, ArH), 4.97(t, *J* 7.6 Hz, 2H), 4.89–4.81 (m, 3H), 4.80 (d, *J* 12.5 Hz, 1H), 4.76 (d, *J* 12.3 Hz, 1H), 4.09 (t, *J* 8.0 Hz, 1H), 3.77 (d, *J* 0.4 Hz, 1H), 3.77–3.64 (m, 1H), 3.63 (s, 3H, -OCH_3), 3.63 (dd, *J* 9.8, 3.4 Hz, 1H), 3.53–3.50 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.3, 151.6, 138.6, 138.5, 138.3, 128.7, 128.6, 128.5, 128.4, 128.1, 127.9, 127.8, 127.8, 118.4, 114.7, 103.1, 82.3, 79.5, 76.9, 75.6, 75.1, 74.4, 73.6, 72.9, 62.1, 55.8; HR-ESI-MS (*m/z*): $[M+Na]^+$ Calcd. for $C_{34}H_{36}NaO_7$ 579.62956, Found: 579.6293.

*Phenyl-6-O-tert-butyldiphenylsilyl-1-thio-β-D-galactopyranoside (**14**):*

Compound **9** (1.55 g, 5.69 mmol) was dissolved in dry acetonitrile (15 mL). To the stirred solution, Im (0.85 g, 12.5 mmol) and TBDPSCl (1.8 mL, 6.80 mmol) were added sequentially at 0°C and the reaction mixture was stirred for 1 h. After complete consumption of starting material (confirmed by TLC), solvents were removed under reduced pressure, obtained residue was purified by column chromatography over silica gel (60% ethyl acetate/pet ether) to give compound **14** as white solid (2.6 g, 91%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75–7.65 (m, 4H, ArH), 7.55–7.50 (m, 2H, ArH), 7.43–7.32 (m, 6H, ArH), 7.23–7.18 (m, 3H, ArH), 4.53 (d, *J* 9.7 Hz, 1H, H-1), 4.07 (s, 1H, H-4), 3.92 (d, *J* 5.2 Hz, 2H, H-6′ and 6″), 3.73 (t, *J* 9.2 Hz, 1H, H-2), 3.59–3.54 (m, 2H, H-3 and H-5), 1.05 (s, 9H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 135.8, 135.7, 133.0, 132.9, 132.1, 130.0, 129.1, 127.9, 127.8, 88.8, 78.5, 75.1, 70.0, 69.7, 63.9, 26.9, 19.3; HR-ESI-MS (*m/z*): $[M+Na]^+$ Calcd. for $C_{34}H_{34}NaO_7$ 577.6295, Found: 577.6293.

*Phenyl-3,4-O-(isopropilidene)-1-thio-β-D-galactopyranoside (**16**):*

To the solution of **9** (2.5 g, 9.18 mmol) in 2, 2-dimethoxy propane (20 mL), dry *p*-TSA (158 mg, 0.92 mmol) was added and stirred up to 3 h at room temperature. The reaction was quenched with Et_3N (0.7 mL). Then toluene (20 mL) was added and the reaction mixture was concentrated *in vacuum* and subsequently co-evaporated with toluene (2×20 mL). To this reaction mixture MeOH:H_2O (10:1, 90 mL) was added and kept for reflux at 70°C. After 5 h, the reaction mixture was co-evaporated with toluene (20×3 mL), obtained residue was by column chromatography on silica gel (80% ethyl acetate/pet ether) to afford **16** (1.54 g, 53%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50–7.53 (m, 2H, ArH), 7.25–7.32 (m, 3H, ArH), 4.47 (d, 1H, *J* 10.1 Hz, H-1), 4.14–4.19 (m, 1H), 4.09 (t, *J* 5.6 Hz, 1H), 3.95 (dd, *J* 7.2 and 11.5 Hz, 1H), 3.79 (dd, *J* 3.8 and 11.5 Hz, 1H), 3.55 (dd, *J* 6.9 and 10.1 Hz, 1H), 2.85 (bs, 2H, OH), 1.40 (s, 3H, CH_3), 1.31 (s, 3H, CH_3); ^{13}CNMR (100 MHz, $CDCl_3$): δ 132.3, 132.0, 129.0, 128.0, 110.0, 87.0, 79.0, 73.0, 71.0, 62.0, 28.0, 26.0; HRMS Calcd. for $C_{15}H_{20}NaO_5S$ $[M+Na]^+$ 335.3813, Found: 335.3828.

*Phenyl-2,6-di-O-benzoyl-3,4-O-(isopropilidene)-1-thio-β-D-galactopyranoside (**17**):*

Compound **16** (1.78 g, 5.7 mmol) was dissolved in CH_2Cl_2 (20 mL) and dry pyridine (2.75 mL, 34.2 mmol) was added at room temperature. To that stirred solution BzCl (3.97 mL, 34.2 mmol) was added at 0°C and kept it for 1 h. Then the reaction mixture was diluted with CH_2Cl_2 and 2(*N*) HCl (5 mL) was added to quench pyridine and washed with $NaHCO_3$. Separated aqueous layer again washed with CH_2Cl_2. Then the combined organic layer was dried over Na_2SO_4 and concentrated *in vacuum*. Chromatography on silica gel (12% ethyl acetate/petroleum ether) afforded **17** as a white solid (2.96 g, 53%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02–8.01 (m, 4H, ArH), 7.57–7.49 (m, 2H, ArH), 7.42–7.37 (m, 6H, ArH), 7.19–7.07 (m, 2H, ArH), 7.01–6.98 (m, 2H, ArH), 5.27 (dd, *J* 10.0, 6.9 Hz, 1H, H-2), 4.73 (d, *J* 10.0 Hz, 1H, H-1), 4.67 (dd, *J* 11.3, 3.6 Hz, 1H, H-6a), 4.56 (dd, *J* 11.3, 8.3 Hz, 1H, H-6b), 4.34 (dd, *J* 6.9, 5.5 Hz, 1H, H-3), 4.28 (dd, *J* 5.4, 2.0 Hz, 1H, H-5), 4.19–4.15 (m, 1H, H-4), 1.55 (s, 3H, -CH_3), 1.30 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.5, 165.6, 133.8, 133.4, 131.9, 130.0, 129.9, 129.88, 129.7, 128.9, 128.6, 128.5, 127.7, 111.2, 86.1, 76.9, 74.6, 73.8, 72.0, 64.3, 27.8, 26.5; HRMS Calcd. for $C_{29}H_{28}NaO_7S$ $[M+Na]^+$ 543.1448, Found: 543.1443.

*Phenyl-2,6-di-O-benzoyl-1-thio-β-D-galactopyranoside (**18**):*

Compound **17** (0.5 g, 0.96 mmol) was dissolved in 80% AcOH (3 mL) and kept the reaction mixture at 80°C for 3 h. After completion of reaction (confirmed by TLC) the reaction mixture was diluted with ethyl acetate and obtained organic layer was washed with $NaHCO_3$ solution. Separated organic layer was dried over Na_2SO_4, concentrated and purified by column chromatography (30% ethyl acetate/pet ether) to give **18** as white solid (0.46 g, 95%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07–8.03 (m, 4H, ArH), 7.59–7.57 (m, 2H, ArH), 7.47–7.42 (m, 6H, ArH), 7.26–7.20 (m, 1H, ArH), 7.18–7.10 (m, 2H, ArH), 5.29 (t, *J* 9.56 Hz, 1H, H-2), 4.84 (d, *J* 10 Hz, 1H, H-1, β), 4.69 (dd, *J* 11.7, 10 Hz, 1H, H-6), 4.62 (dd, *J* 25.3, 7.4 Hz, 1H, H-6′), 4.11 (s, 1H, H-4), 3.94 (t, *J* 6.8 Hz, 1H, H-5), 3.88 (dd, *J* 9.16, 2.5 Hz, 1H, H-3), 3.06 (bs, 2H, OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.1, 166.7, 133.7, 133.5, 132.3, 130.2, 129.9, 129.8, 129.6, 129.02, 128.6, 27.9, 86.5, 76.5, 73.8, 72.2, 69.2, 63.8; HRMS Calcd. for $C_{26}H_{24}NaO_7S$ $[M + Na]^+$ 503.1138, Found: 503.1333.

*Phenyl-2,6-di-O-benzoyl-3,4-O-[1-(methoxycarbonyl)-ethylidene]-1-thio-β-D-galactopyranoside (**19**):*

Compound **18** (2.05 g, 4.27 mmol) was dissolved in methyl pyruvate (9.3 mL, 102.68 mmol). To this stirred solution, $BF_3 \cdot OEt_2$ (1.07 mL, 8.53 mmol) was added in dropwise manner at room temperature and the reaction mixture was kept for 1 h. After complete consumption of starting material (confirmed by TLC), triethylamine was added to quench $BF_3 \cdot OEt_2$ and the reaction mixture was diluted in CH_2Cl_2 and obtained organic layer was washed with $NaHCO_3$ solution. Then separated organic layer was dried over Na_2SO_4, concentrated, obtained residue was purified by column chromatography over silica gel (30% ethyl acetate/pet ether) to give diastereomeric compound **19** (*R*:*S* = 3.3:1, 2.04 g, 84%) as white solid. The obtained compound was characterized by various spectroscopic methods, For R isomer: $[\alpha]_D$ = +19.75 (*c* = 0.8, chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (d, *J* 0.4 Hz, 4H, ArH), 7.61–7.58 (m, 2H, ArH), 7.56–7.43 (m, 6H, ArH), 7.16–7.14 (m, 1H, ArH), 7.07–7.04 (m, 2H, ArH), 5.54 (dd, *J* 10.4, 7.2 Hz, 1H, H-2), 4.80–4.76 (m, 2H, H-1, H-6′), 4.69–4.59 (m, 2H, H-3, H-6), 4.41 (dd, *J* 5.4, 2.0 Hz, 1H, H-4), 4.26 (ddd, *J* 3.7, 2.1, 2.1 Hz, 1H, H-5), 3.86 (s, -$COOCH_3$), 1.58 (s, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.3, 166.2, 165.2, 133.5, 133.4, 133.3, 131.8, 129.9, 129.74, 129.69, 128.9, 128.8, 128.4, 127.8, 127.7, 106.8, 86.1, 78.5, 76.9, 75.5, 74.8, 74.33, 73.9, 71.9, 70.3, 63.9, 52.9, 52.6, 23.2, 22.9; HRMS Calcd. for $C_{30}H_{28}NaO_9S$ $[M+Na]^+$ 587.1346, Found: 587.1330.

*4-Methoxyphenyl-2,6-di-O-benzoyl-3,4-O-[1-(methoxycarbonyl)ethylidine]-β-(1→6)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (**20**):*

Bromine (2.6 μL, 0.90 mmol) was added to a clear solution of compound **19** (0.2 g, 0.35 mmol) in CH_2Cl_2 (5 mL) at 0°C. After 1 h, toluene was added, the mixture was concentrated and the residue was co-evaporated twice with toluene.

A solution of acceptor **5** (0.12 g, 0.21 mmol) in CH_2Cl_2 (2.5 mL) was added to a suspension of glycosyl bromide, 3 Å MS (0.2 g) and sym. collidine (8.7 μL, 0.64 mmol) in CH_2Cl_2 (2.5 mL) and kept stirring at RT for 30 min. Then, AgOTf (0.18 g, 0.71 mmol) was added and stirring was continued at the same temperature. After 2 h, triethylamine (0.1 mL) was added and the reaction mixture was diluted with CH_2Cl_2, filtered through celite, and concentrated. The residue was puri-

fied by column chromatography (40% ethyl acetate/pet ether) to give **20** as a viscous liquid (0.24 g, 61%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17–8.10 (m, 2H, ArH), 8.05–8.01 (m, 2H, ArH), 7.78–7.69 (m, 3H, ArH), 7.67–7.55 (m, 7H, ArH), 7.40–7.28 (m, 5H, ArH), 7.28–7.14 (m, 7H, ArH), 7.04 (dd, *J* 6.8, 2.1 Hz, 2H, ArH), 6.85 (dd, *J* 7.0, 2.2 Hz, 2H, ArH), 5.67 (d, *J* 5.2 Hz, 1H, H-2), 5.02 (dd, 2H, *J* 11.1, 3.7 Hz, 2H, H-6a, 6b), 4.90–4.85 (m, 6H, CH_2Ph), 4.85–4.80 (m, 2H, H-3, 5), 4.74 (d, *J* 9.4 Hz, 1H, H-1), 4.57 (d, *J* 9.3 Hz, 1H, H-1′), 4.51 (t, *J* 4.2 Hz, 1H, H-4), 4.07 (dd, *J* 7.6, 2.7 Hz, 1H, H-2′), 3.84 (s, 3H, -$COOCH_3$), 3.835 (s, 3H, -OCH_3), 3.83 (t, *J* 1.8 Hz, 1H, H-4′), 3.80–3.79 (m, 1H, H-5′), 3.59 (dd, *J* 9.7, 2.8 Hz, 1H, H-3′), 3.42–3.39 (m, 2H, H-6a′, 6b′), 1.65 (s, 3H, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.4, 151.7, 138.5, 133.2, 130.1, 129.9, 129.8, 129.7, 128.6, 128.5, 128.48, 128.4, 128.36, 128.2, 127.8, 127.7, 126.1, 121.5, 118.8, 114.6, 109.01, 103.09, 79.4, 75.5, 74.6, 73.3, 72.2, 70.8, 67.9, 63.8, 55.8, 52.6, 26.7, 24.3, 24.1; HR-ESI-MS (*m/z*): $[M+Na]^+$ Calcd. for $C_{58}H_{58}NaO_{16}$ 1034.0715 Found: 1034.0722.

4-Methoxyphenyl-(2,3-di-O-acetyl-4,6-benzilidine-β-D-galactopyranoside)-(1→6)-(3,4-O-[1-(methoxycarbonyl)-ethylidine]-β-D-galactopyranoside)-(1→6)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (**22**)*:*

NaOMe (37 mg, 0.70 mmol) was added to a stirred solution of compound **20** (0.24 g, 0.23 mmol) in MeOH (5 mL). Then the mixture was stirred for 1/2 h. After complete conversion of starting material reaction mixture was diluted with MeOH, concentrated and purified compound by column chromatography (60% ethyl acetate/pet ether) to give compound **21** as a white solid (20 mg, 70%).

An azetroped mixture of donor **3** (27 mg, 0.06 mmol), acceptor **21** (30 mg, 0.03 mmol) and 3 Å MS (100 mg) were added in CH_2Cl_2 (2 mL) and stirred for 0.5 h at rt. After 0.5 h, NIS (28 mg, 0.12 mmol) and TMSOTf (1.0 μL, 0.006 mmol) were added at –30°C and reaction mixture was kept for 1 h. After completion of reaction, Et_3N was added and filtration of molecular sieves was done by celite and washed with CH_2Cl_2. Obtained filtrate was washed with aq. $Na_2S_2O_3$ and brine solution. Separated organic layer was dried over anhydrous Na_2SO_4, concentrated. Obtained residue was purified by column chromatography over silica gel (40% ethyl acetate/pet ether) to give trisaccharide **22** (27 mg, 78%) as white viscous liquid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55–7.42 (m, 3H, ArH), 7.40–7.30 (m, 19H, ArH), 7.01 (d, *J* 2.2 Hz, 2H, ArH), 6.85 (d, *J* 9.1, 2.0 Hz, 2H, ArH), 5.50 (s, 1H, -PhCH), 5.38 (t, *J* 2.7, 2 Hz, 2H), 5.33 (dd, *J* 10.3, 7.9 Hz, 1H), 5.12–5.09 (m, 2H), 4.99 (dd, *J* 13.6, 10.9 Hz, 1H), 4.92 (dd, *J* 14.1, 3.6 Hz, 1H), 4.80–4.71 (m, 6H, -CH_2Ph), 4.57 (d, *J* 8.0 Hz, 1H), 4.51 (d, *J* 12.6 Hz, 1H, H-1), 4.46 (t, *J* 9.0 Hz, 1H), 4.34 (dd, *J* 12.6, 5.5 Hz, 2H), 4.08 (dd, *J* 11.0, 7.2 Hz, 1H), 4.02–3.91 (m, 1H), 3.81 (dd, *J* 5.6, 3.4 Hz, 1H), 3.77 (s, 3H, -$COOCH_3$), 3.74 (s, 3H, -OCH_3), 3.62 (t, *J* 4.2 Hz, 1H), 3.57 (dd, *J* 9.8, 2.9 Hz, 1H), 3.46–3.34 (m, 2H), 3.45 (d, *J* 9.5 Hz, 1H), 2.10 (s, 3H, -OAc), 2.03 (s, 3H, -OAc), 1.56 (s, 3H, -CH_3); HR-ESI-MS (*m/z*): $[M+Na]^+$ Calcd. for $C_{61}H_{68}NaO_{21}$ 1159.4151, Found: 1159.4163.

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