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Total synthesis of the bacillosamine containing α-L-serine linked trisaccharide of *Neisseria meningitidis*

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ABSTRACT

Total synthesis of the bacillosamine containing L-serine linked *O*-trisaccharide of *Neisseria meningitidis* is described. The synthesis entails installation of two consecutive α-linkages including the coupling of bacillosamine with L-serine derivative.

1. Introduction

Neisseria meningitidis (menigococcus) is a causative bacterium of the highly contagious disease meningitis which involves inflammation of the protective membranes (meninges) of the brain and spinal cord.[1] Meningitis is most common in children aged 2–18 and has a high mortality rate. It is estimated that about 5–10% of the total population may be asymptomatic carriers. Most cases are acquired through the exposure to these carriers. The onset of symptoms is sudden and death can occur in a few hours. In a meningitis pandemic, about 10% of patients die, while about 15% of the survivors develop serious neurological disorders. Thus, novel and more effective vaccines[2] are required to control the periodic outbreak of this deadly disease.

It has been well established that meningococcus pili, which are long polymeric filamentous glycoproteins produced from the surface of pathogenic *N. meningitidis* are a key virulence factor.[3] Pili play crucial roles as essential adhesins in colonization of this capsular bacterium and contribute to the specificity for the human host.[4] In 1995, Stimson et al.[5] showed that the pili are post-translationally modified by glycosylation of serine 63 with a unusual trisaccharide Gal-(β 1-4)-Gal(α 1-3)-2,4-diacetimido-2,4,6-trideoxyhexose [Gal(β 1-4)Gal(α 1-3) DATDH] (Fig. 1). Since the structure of the trisaccharide was proposed based on the linkage analysis by acid hydrolysis and mass spectroscopic studies, the stereochemistry at C4 of the rare sugar (DATDH) could not be defined.

The specific function of glycosylation in meningococcus infection remains obscure.[6] It is suggested that glycosylation may constitute bacterial cloaking devices against the host immune reponses.[7] More importantly, Marceau and Nassif demonstrated that the presence of the glycosylation at serine 63 can influence the amount of soluble and secreted form of pilin (S-pilin), a molecule which is crucial in establishing infections.[8] Since the pilin *O*-glycan contains unique deoxy amino sugars which are not present on the host cell surfaces, the structural differences can be exploited for the development of target specific therapeutics and vaccines.[9]

Given the biological importance and the problems associated with the isolation of the glycoproteins in acceptable amounts and purity, it is imperative to synthesize the serine-linked trisaccharide that can be incorporated into the glycoprotein to expedite the studies probing the exact role of pilin *O*-glycans in meningitis and further vaccine development.

The structure of the target *O*-trisaccharide comprises a digalactosyl moiety, a rare deoxy amino sugar DATDH and L-serine. Since the stereochemistry at C4 of the rare sugar is not defined, two putative trisaccharides **1** and **2** are possible, one with a 2,4-diacetamido 2,4,6-trideoxy D-galactose (DATDG), and one containing a 2,4-diacetamido 2,4,6-trideoxy D-glucose (bacillosamine, Bac), respectively. The main challenges involved in the synthesis of the deceptively simple looking trisaccharides are the synthesis of the rare, deoxyamino glycans[10–13] (Bac[11] and DATDG[12]) and installation of two consecutive α-glycosyl linkages. Moreover, obtaining

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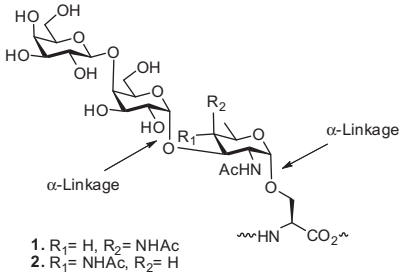


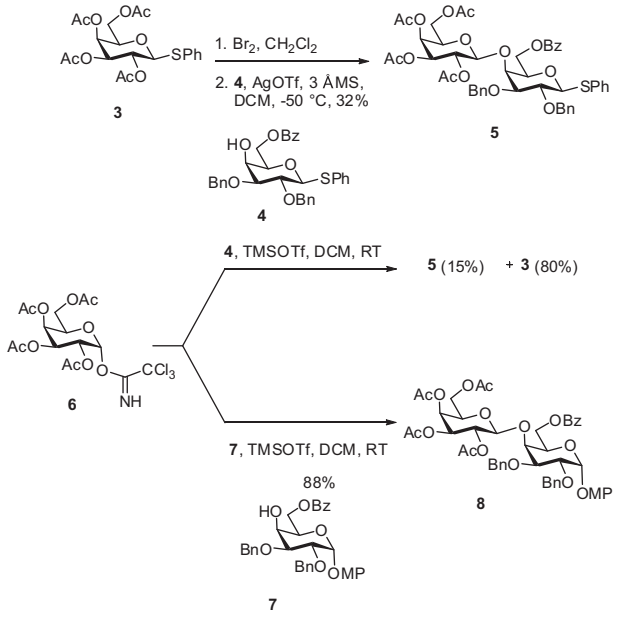
Figure 1. Structures of pilin glycans of *Neisseria meningitidis*.

complete α-stereoselectivity in the coupling of L-serine with the rare trideoxy amino sugars is difficult. Towards this goal, we recently established a methodology to access the rare bacterial deoxy amino sugars including DATDH and thereby accomplished the first synthesis of trisaccharide **1**.[14] In this article we describe the total synthesis of the bacillosamine containing trisaccharide **2**.

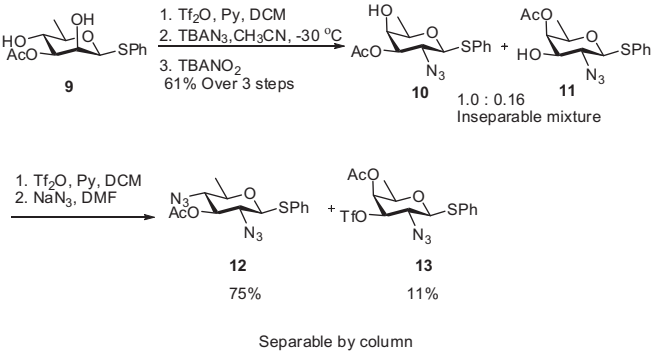
2. Results and discussions

2.1. Synthesis of the left hand disaccharide

For the synthesis of the left hand disaccharide (di-gal), we first prepared the known thioglycoside donor **3**[15] and acceptor **4**[16] using reported procedures. First, thioglycoside **3** was treated with bromine in CH_2Cl_2 and the so formed glycosyl bromide was activated with AgOTf and orthogonally coupled with acceptor **4** to afford the desired disaccharide **5**, albeit in a modest 32% yield (Scheme 1). The low yield encountered in this reaction was attributed to the simultaneous formation of the corresponding orthoester[17] which could not be rearranged to **5** even after prolonged stirring in the presence of excess TMSOTf. Since, we were not able to improve the yield of **5** using glycosyl bromide under various conditions, we decided to try out the corresponding known imidate derivative **6**,[18] with the hope to circumvent the orthoester formation. Glycosylation of imidate **6** with **4** using TMSOTf as an activator at rt[19] did furnish disaccharide **5**, but again as a minor



Scheme 1. Synthesis of the left hand side disaccharide unit.



Scheme 2. Synthesis of bacillosamine thioglycoside donor **12**.

product (15%). In this case, although we did not encounter the orthoester, instead thioglycoside **3** was obtained as a major side product (80%), formed presumably via the aglycon transfer of SPh from acceptor **4** to donor **6**. Such aglycon transfers of thioglycosides are well documented in the literature.[20] To obviate the aglycon transfer, the anomeric thiophenyl group was replaced by a stable methoxy phenyl group (OMP). For this purpose, the corresponding OMP glycoside **7** was prepared in a manner very much similar to **4** from the known α-OMP galactoside[21] through sequential 4,6-O-benzylidenation ($PhCH(OMe)_2$, CSA), 2,3-di-O-benzylation (NaH, BnBr), benzylidene hydrolysis (80% AcOH reflux) and selective O6 benzoylation (Et_3N, Bz_2O), in 68% overall yields. Gratifyingly, glycosylation of imidate **6** with acceptor **7** under TMSOTf activation at rt furnished the desired β-linked disaccharide **8** in 88% yields.[14b]

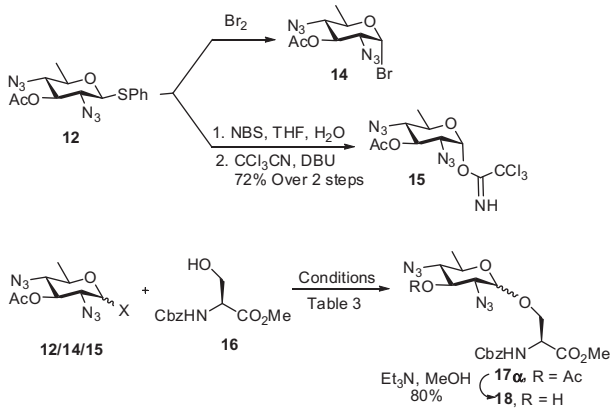
2.2. Synthesis of the Bac building block

The rare Bac building block **12** was prepared from **9** following our recently established protocol to synthesize rare bacterial sugars (Scheme 2).[14] For this purpose, we were required to carry out a double inversion at C4 of a D-rhamnose derivative to achieve azide substitution with retention of configuration. First, diol **9** was subjected to triflation followed by a one-pot double serial inversion which involved a highly regioselective azide displacement of the C2-OTf followed by a nitrite ion mediated displacement (Lattrell-Dax reaction[22]) of the remaining C4-OTf. The reaction smoothly delivered the D-fucosamine derivative **10** accompanied by a small amount of acetate migration product **11** in a ratio 1:0.16 (as judged by ^1H NMR). Since this side product was inseparable on TLC and by column chromatography, the mixture was subjected as such to a similar C4-triflation and azide displacement sequence, to afford the desired Bac derivative **12** in 75% isolated yield over two steps. At this stage, the unreacted C3-OTf **13** (well separated on TLC) was isolated in 11% yield.

2.3. Stereoselective glycosylation of bacillosamine donor with L-serine acceptor

With the appropriate building blocks in hand, we turned our attention to the synthesis of the right hand unit. As anticipated, the installation of 1,2-*cis* glycosidic linkage in the coupling of the 6-deoxy monosaccharide with the primary OH of L-serine was very difficult. In this case, although the C2-azido group, being a non-participating group, is expected to facilitate the formation of α-linkage, remote participation is not available from the C4 and C6 functionalities.[23] So, we were mindful that various donors and conditions may need to be tested to achieve selectivity. Strategically, our rare sugar building blocks being stable thioglycosides,

Table 1
Stereoselective coupling of Bac donors with L-serine acceptor



Entry	Donor	Promotor	Temp (°C)	Solvent	Time (h)	α/β	Yield (%)
1	Imidate **15**	TMSOTf	−78	THF	4	1.8/1	93
2	Thioglycoside **12**	Ph_2SO/Tf_2O	−60	CH_2Cl_2	1	2.0/1.0	78
3[a]	Bromide **14**	TBAI	rt	CH_2Cl_2	10	1.0/0	52

[a] Yield over 2 steps.

could be easily transformed into other donors such as halides and imidates.[24] Thus, reaction of **12** with Br_2 in dichloromethane generated the corresponding glycosyl bromide **14**, whereas treatment of **12** with aqueous NBS generated the corresponding anomeric hemiacetals, which were readily converted into imidate **15** (Table 1).

Earlier, we were successful in achieving a complete α-stereoselectivity in the glycosylation of a 3-*O*-AcCl, 4-*O*-Ac, 6-*O*-TBDPS D-GalN_3 thioglycoside[25] with L-serine derivative **16**, using diphenyl sulfoxide and Tf_2O combination, essentially under Boons' conditions.[26] Subsequently we found that these reaction conditions did not work well for DATDH and after screening several donors and reaction conditions, we were able to achieve excellent α-selectivity using trichloroacetimidate donor under TMSOTf activation conditions and using THF as a participating solvent[27] at −78 °C.[14b] In continuation of these studies, we employed the same conditions for glycosylation of the bacillosamine derivative **12**. To our dismay, the corresponding imidate **15** under the same conditions of solvent participation did not give good selectivity (Table 1, entry 1). The desired product **17** was obtained as 1.8:1 α:β mixture (93%) which was inseparable on TLC. Activation of thioglycoside **12** using Ph_2SO/Tf_2O combination as promoter also showed similar results (Table 1, entry 2). For practical purpose, since the isomers could not be separated by column chromatography, it was necessary to get only α-product in this glycosylation. The exclusive α-selectivity was finally achieved using glycosyl bromide under in situ anomerization conditions.[28] Accordingly, thioglycoside **12** was converted into corresponding α-bromide **14**, which underwent a facile coupling with acceptor **16** in the presence of TBAI to afford the desired α-isomer **17** in 52% yields over 2 steps (Table 1, entry 3). Selective removal of the acetyl group in **17** using Et_3N and methanol combination revealed 3-OH acceptors **18** (80%).

2.4. Synthesis of the L-serine linked *O*-trisaccharides

With the left hand disaccharide unit **8** and right hand glycosyl amino acid acceptor **18** in hand, the stage was now set for the crucial [2+1] relay glycosylation. First the OMP glycoside was hydrolysed by using ceric ammonium nitrate (CAN) and the so formed anomeric hemiacetal was smoothly converted into an imidate **19** (76% over 2 steps). Unfortunately, glycosylation of imidate **19** with the acceptor **18** under TMSOTf activation failed to give any coupling product (Scheme 3). The imidate was too reactive and spontaneously decomposed even at −78 °C. Use of AgOTf[29] in place of TMSOTf at −50 °C also gave similar results. So, we resorted to the corresponding glycosyl chlorides which could be generated from the OMP glycosides.[30] A combination of AcCl, $BF_3{\cdot}OEt_2$ and cat. ZnI_2 converted the α-anomeric OMP in **8** into chloride to afford the donor **20** in 72% yield.[14b] Chloride **20** underwent a facile AgOTf promoted glycosylation with the Bac acceptor **18**, to afford exclusively α-linked product **21** (^{1}H NMR δ 4.76, d, $J_{1',2'}$ = 3.7 Hz, H-1′), in 82% yield (Scheme 3). The α-selectivity in this case is presumably arising through the intermediacy of the a highly reactive glycosyl β-triflate or more likely a solvent separated contact ion pair,[28c] which undergoes a nucleophilic displacement with acceptor **18** from α-face to afford the desired trisaccharide **21** with exclusive α-selectivity.

Deprotection of **21** involving Staudinger reduction of azide generated the corresponding amine, which was treated with acetic anhydride to afford the desired *N*-acetylated product **22** in 72% yields. Then benzyl groups were oxidatively[31] removed in the presence of Cbz group under Iadonisi's conditions using $NaBrO_3$ and $Na_2S_2O_4$ to obtain the diol **23** in 85% yield. Finally de-O-acetylation and simultaneous hydrolysis of methyl ester[32] afforded the target molecule **24**, in 89% yield.

3. Conclusion

Bacterial glycoproteomics is an emerging area of research.[9,33] Synthesis of the *O*-glycans of *N. meningitidis* is a crucial step towards understanding the role of pilin glycosylation in pathogenesis. We have carried out the first total synthesis of the bacillosamine containing L-serine linked trisaccharide present on the pili of *N. meningitidis* in an efficient manner. Final compound **24** is well suited for incorporation into the protein for the synthesis of the glycoprotein which can be further used for studying the biological role of the pilin glycans. The total synthesis entails two consecutive α-glycosylations involving the bacterial rare deoxy amino sugar Bac. In this endeavour, we also studied for the first time the coupling of bacillosamine with L-serine derivative and established the conditions to obtain exclusive α-selectivity. These studies will be useful for the synthesis of other Bac containing bacterial glycans.

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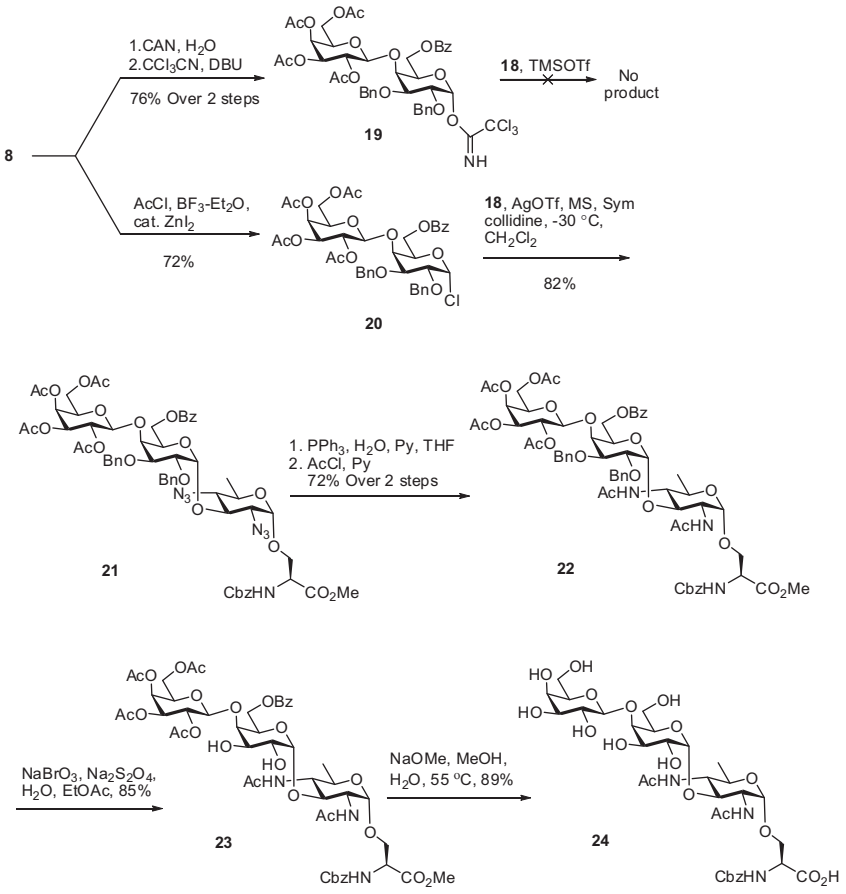

Scheme 3. Synthesis of the trisaccharide **24**.

4. Experimental section

4.1. General methods

All reactions were conducted under a 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 were oven dried before use. TLC was performed on pre-coated Aluminum 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 solution. Silica gel column chromatography was performed using Silica Gel (100-200 mesh) and employed a solvent polarity correlated with TLC mobility. NMR experiments were conducted on 400 MHz instrument using $CDCl_3$ (D, 99.8%) or $(CD_3)_2CO$ (D, 99.9%) or CD_3OD (99.8%) as solvents. Chemical shifts are relative to the deuterated solvent peaks and are in parts per million (ppm). ^1H–^1H COSY was used to confirm proton assignments. Mass spectra were acquired in the ESI mode using Q-TOF analyser. Specific rotation experiments were measured at 589 nm (Na) and 20 °C. IR spectra were recorded on an FT-IR spectrometer using CsCl plates.

4.2. Phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galctopyranosyl-(1→4)-6-*O*-benzoyl-2,3-dibenzyl-1-thio-α-D-galactopyranoside (5)

4.2.1. Through glycosyl bromide

Bromine (0.09 mL, 1.7 mmol) was added to a solution of **3**[15] (0.35 g, 0.79 mmol) in CH_2Cl_2 (9 mL). After 30 min, toluene was added, the mixture was concentrated and the residue was co-evaporated twice with toluene.

The residue which was obtained after solvent removal was dissolved in CH_2Cl_2 (3 mL) and added to a solution of acceptor **4**[16] (0.0.28 g, 0.5 mmol) in CH_2Cl_2 (3 mL) containing 3 Å MS (0.6 g). The mixture was stirred under nitrogen for 30 min at rt, after which the temperature was lowered to −50 °C and AgOTf (0.19 g, 0.75 mmol) was added. After 2 h Et_3N was added, and the stirring was continued for 10 min. The mixture was diluted with CH_2Cl_2, filtered through celite and concentrated. The residue was purified by silica gel chromatography (20% ethyl acetate/petroleum ether) to give the desired product **5** as a yellowish liquid (0.17 g, 32%).

4.2.2. Through imidate

TMSOTf (0.03 mL, 0.16 mmol) was added drop wise to a solution of imidate **6**[18] (0.39 g, 0.79 mmol), acceptor **4**[16] (0.3 g, 0.55 mmol) and 3 Å MS (0.6 g) at rt and the reaction mixture was allowed to stir at the same temperature for 4 h. After 4 h the mixture was diluted with CH_2Cl_2, filtered through celite and concentrated. The residue was purified by silica gel chromatography (20% ethyl acetate/petroleum ether) to give the desired product **5** as a yellowish liquid (0.07 g, 15%): $[\alpha]^{20}_D$ +8.9 (*c* 0.31, $CHCl_3$); IR ($CHCl_3$) *ν* 3020, 2927, 1751, 1422, 1216, 1048, 669 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 8.08 (d, *J* = 7.0 Hz, 2H, ArH), 7.63–7.28 (m, 15H, ArH), 7.19–7.10 (m, 3H, ArH), 5 .37 (d, *J* = 3.2 Hz, 1H, H-4′), 5.28 (ap.t, *J* = 10.0, 7.8 Hz, IH, H-2′), 5.05 (dd, *J* = 10.0, 3.2 Hz, 1H, H-3′), 4.90–4.62 (m, 7H, H-1, H-1′, H-6a, ArH), 4.53–4.48 (m, 1H, H-6b), 4.12 (d, *J* = 6.8 Hz, 2H, H-6′), 4.05 (d, *J* = 3.0 Hz, 1H, H-4), 3.82 (t, *J* = 6.4, Hz, IH, H-5′), 3.78–3.75 (m, 1H, H-5), 3.69 (t, *J* = 10.0, Hz, IH, H-2), 3.58 (dd, *J* = 10.0, *J* = 3.0 Hz, 1H, H-3), 2.18 (s, 3H, CH_3), 2.03 (s, 6H, CH_3), 1.92

(s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ170.6, 170.5, 170.4, 169.5, 166.5, 138.1, 137.9, 134.7, 133.3, 131.3, 130.1, 129.8, 129.7, 128.9, 128.7, 128.6, 128.4, 128.3, 128.1, 127.2, 102.2, 88.5, 83.2, 77.8, 76.1, 75.9, 75.5, 73.7, 71.0, 70.5, 69.1, 67.0, 64.9, 61.5, 21.0, 20.85, 20.82, 20.8; HR-ESI-MS (*m*/*z*): $[M+Na]^+$ calcd for $C_{47}H_{50}O_{15}SNa$, 909.2768; found, 909.2761.

4.3. Phenyl 2-azido-3-*O*-acetyl-2,6-dideoxy-1-thio-β-D-galactopyranoside (10)

Tf_2O (11.4 mL, 67.5 mmol) and pyridine (11.8 mL, 146.4 mmol) were added sequentially at −10 °C to a stirred solution of **9** (3.36 g, 11.2 mmol) in CH_2Cl_2 (150 mL). Then the reaction mixture was gradually warmed to 10 °C over 2 h. After complete consumption of the starting material, the reaction mixture was diluted with CH_2Cl_2 and washed successively with 1 M HCl, aq. $NaHCO_3$ and brine. Separated organic layer was dried over Na_2SO_4 and concentrated.

The crude product which was obtained after removal of solvents was dissolved in acetonitrile (200 mL) and to this, $TBAN_3$ (3.1 g, 10.0 mmol) was added at −30 °C and the reaction was stirred at the same temperature for 20 h. $TBANO_2$ (4.8 g, 16.9 mmol) was added and the reaction mixture stirred at rt for 1 h. The reaction mixture was diluted with EtOAc and washed with water. Separated organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography on silica gel (1:9 ethyl acetate/pet ether) to obtain **10** as a pale yellowish viscous liquid (2.3 g, 61%): ^1H NMR (400 MHz, $CDCl_3$) δ 7.62–7.58 (m, 2H, ArH), 7.37–7.31 (m, 3H, ArH), 5.12 (d, *J* = 3.0 Hz, 1H, H-4 of **11**), 4.79 (dd, *J* = 10.0, 3.0 Hz, 1H, H-3), 4.46 (d, *J* = 10.0 Hz, 1H, H-1), 3.85 (d, *J* = 3.0 Hz, 1H, H-4), 3.74–3.63 (m, 2H, H-2 & H-5), 3.50 (t, *J* = 10.0 Hz, 1H, H-2 of **11**), 2.15 (s, 3H, CH_3), 1.42 (d, *J* = 6.4 Hz, 3H, CH_3 of **11**), 1.33 (d, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 133.5, 133.2, 131.4, 129.2, 129.0, 128.6, 128.3, 86.5, 76.0, 74.7, 73.4, 73.0, 72.2, 69.3, 62.5, 59.4, 21.12, 16.9, 16.7; HR-ESI-MS (*m*/*z*): $[M+Na]^+$ calcd for $C_{14}H_{17}N_3O_4NaS$, 346.0832; found, 346.0827.

4.4. Phenyl 2,4-diazido-3-*O*-acetyl-2,4,6-trideoxy-1-thio-β-D-glucopyranoside (12)

Tf_2O (0.9 mL, 5.38 mmol) was added drop wise at −10 °C to a stirred solution of **10** (1.45 g, 4.48 mmol) and pyridine (2.2 mL, 26.9 mmol) in CH_2Cl_2 (20 mL) and this solution was gradually brought to 10 °C over 2 h. After complete consumption of starting material, reaction mixture was concentrated in vacuo and the crude product was used for the next step without purification.

The crude product which was obtained in above step was dissolved in DMF (30 mL) and to this, NaN_3 (2.9 g, 44 mmol) was added. The reaction mixture was stirred at rt for 10 h and then it was diluted with EtOAc and washed with water. Separated organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography (10% ethyl acetate/pet ether) to afford **12** as a white solid (1.17 g, 75%) and in this reaction **13** was obtained as side product in 11% yield. Data of **12**: $[\alpha]^{20}_D$ −41.5 (*c* 0.61, $CHCl_3$); IR ($CHCl_3$) *ν* 3020, 2927, 2111, 1759, 1216, 1047, 669 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.56–7.54 (m, 2H, ArH), 7.35–7.32 (m, 3H, ArH), 5.02 (t, *J* = 10.0 Hz, 1H, H-3), 4.47 (d, *J* = 10.0 Hz, 1H, H-1), 3.36–3.28 (m, 2H, H-2 & H-5), 3.12 (t, *J* = 10.0 Hz, 1H, H-4), 1.38 (d, *J* = 6.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 133.8, 130.8, 129.2, 128.8, 85.9, 75.0, 74.6, 65.4, 63.1, 20.8, 18.6; HR-ESI-MS (*m*/*z*): $[M+Na]^+$ calcd for $C_{14}H_{16}N_6O_3NaS$, 371.0897; found, 371.0889; Data of **13** (pale yellowish viscous liquid): $[\alpha]^{20}_D$ −36.8 (*c* 1.83, $CHCl_3$); IR ($CHCl_3$) *ν* 2988, 2855, 2117, 1755, 1419, 1245, 1220, 1142, 1077, 892, 749, 693, 617 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.64–7.62 (m, 2H, ArH), 7.37–7.33 (m, 3H, ArH), 5.36 (d, *J* = 3.0 Hz, 1H, H-4), 4.68 (dd, *J* = 10.0, 3.0 Hz, 1H, H-3), 4.50 (d, *J* = 10.0 Hz, 1H, H-1), 3.76–3.70 (m, 2H, H-2 & H-5), 2.13 (s, 3H, CH_3), 1.26 (d, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 133.9, 130.5, 129.2, 129.0, 120.0, 116.8, 86.7, 85.3, 73.2, 69.8, 59.8, 20.4, 16.7; HR-ESI-MS (*m*/*z*): $[M+Na]^+$ calcd for $C_{15}H_{16}F_3N_3O_6NaS_2$, 478.0325; found, 478.0322.

4.5. 3-*O*-Acetyl-2,4-diazido-2,4,6-trideoxy-α-D-glucopyranosyl trichloroacetimidate (15)

NBS (1.0 g, 6.0 mmol) was added at 0 °C to a cooled solution of **12** (0.7 g, 2.0 mmol) in THF/H_2O (30 mL, 4:1). After 10 min. reaction mixture was brought to rt and stirred for 30 min. Then solvents were evaporated and the crude product was purified by column chromatography on silica gel (20% ethyl acetate/pet ether) to afford the desired hemiacetal as a viscous liquid (0.45 g, 88%).

DBU (70 μL, 0.47 mmol) was added at −5 °C to the solution of hemiacetal (0.4 g, 1.56 mmol) and Cl_3CCN (1.9 mL, 19.0 mmol) in CH_2Cl_2 (10 mL) and the reaction mixture was stirred at the same temperature for 1 h. The mixture was concentrated under reduced pressure and the crude product was purified by column chromatography on silica gel (10% ethyl acetate/pet ether) to afford **15** as a white foam (0.45 g, 72%): ^1H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H, NH), 6.39 (d, *J* = 3.5 Hz, 1H, H-1), 5.49 (t, *J* = 10.0 Hz, 1H, H-3), 3.91 (q, *J* = 6.4 Hz, 1H, H-5), 3.59 (dd, *J* = 10.0, 3.5 Hz, 1H, H-2), 3.27 (t, *J* = 10.0 Hz, 1H, H-4), 2.20 (s, 3H, CH_3), 1.35 (d, *J* = 6.4 Hz, 3H).

4.6. *N*-(Benzyloxycarbonyl)-*O*-(3-*O*-acetyl-2,4-diazido-2,4,6-trideoxy-α-D-glucopyranosyl)-L-serine methylester (17)

Bromine (0.12 mL, 0.2.4 mmol) was added to a solution of **12** (0.45 g, 1.09 mmol) in CH_2Cl_2 (14 mL). After 1 h, toluene was added, the mixture was concentrated and the residue was co-evaporated twice with toluene.

The residue in CH_2Cl_2 (2.5 mL) was added to a solution of aminoacid acceptor **16** (0.0.41 g, 0.1.6 mmol), 3 Å MS (0.5 g) and TBAI (1.2 g, 3.3 mmol) in CH_2Cl_2 (2.5 mL). After 8 h the mixture was diluted with CH_2Cl_2, filtered through celite and concentrated. The residue was purified by silica gel chromatography to give the desired product **17** as a pasty white solid (0.28 g, 52% over 2 steps, only α-isomer): $[\alpha]^{20}_D$ +86.6 (*c* 0.74, $CHCl_3$); IR ($CHCl_3$) *ν* 3019, 2928, 2109, 1750, 1714, 1512, 1216, 1042, 669 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.37–7.29 (m, 5H, ArH), 5.75 (d, *J* = 8.0 Hz, 1H, NH), 5.32 (t, *J* = 10.0 Hz, 1H, H-3), 5.13 (s, 2H, CH_2 of Cbz), 4.87 (d, *J* = 3.5 Hz, 1H, H-1), 4.57–4.54 (m, 1H, –CH), 4.01 (dq, *J* = 10.5, 3.1 Hz, 1H, $-CH_2$), 3.78 (s, 3H, CH_3), 3.74–3.63 (m, 1H, H-5), 3.15 (t, *J* = 10.0 Hz, 1H, H-4), 3.07 (dd, *J* = 10.0, 3.5 Hz, 1H, H-2), 2.16 (s, 3H, CH_3), 1.27 (d, *J* = 6.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 169.9, 156.0, 136.2, 128.7, 128.4, 128.3, 98.9, 70.4, 69.0, 67.3, 66.8, 66.2, 61.2, 54.4, 53.1, 20.9, 18.2; HR-ESI-MS (*m*/*z*): $[M+Na]^+$ calcd for $C_{20}H_{25}N_7O_8Na$, 514.1657; found, 514.1658.

4.7. *N*-(Benzyloxycarbonyl)-*O*-(2,4-diazido-2,4,6-trideoxy-α-D-glucopyranosyl)-L-serine methylester (18)

Et_3N (1 mL) was added to a clear solution of **17** (0.2 g, 0.4 mmol) in MeOH (4 mL) and the reaction mixture kept for stirring at rt overnight in the dark. After complete consumption of starting material solvents were removed in vacuo and the crude product was chromatographed by silica gel column chromatography (30% ethyl acetate/pet ether) to afford **18** as a viscous liquid (0.15 g, 80%): $[\alpha]^{20}_D$ +158.5 (*c* 0.19, $CHCl_3$); IR ($CHCl_3$) *ν* 3430, 3020, 2934, 2110, 1721, 1216, 1042, 669 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.37–7.29 (m, 5H, ArH), 5.87 (d, *J* = 8.0 Hz, 1H, NH), 5.12

(s, 2H, CH_2 of Cbz), 4.80 (d, J = 3.4 Hz, 1H, H-1), 4.57–4.55 (m, 1H, –CH), 3.95 (d, J = 2.8 Hz, 2H, –CH_2), 3.94 (t, J = 10.0 Hz, 1H, H-3), 3.77 (s, 3H, CH_3), 3.61–3.51(m, 1H, H-5), 3.15 (dd, J = 10.0, 3.4 Hz, 1H, H-2), 3.04 (t, J = 10.0 Hz, 1H, H-4), 1.25 (d, J = 6.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.37, 156.1, 136.1, 128.7, 128.4, 128.3, 98.5, 70.3, 68.5, 68.7, 67.3, 67.0, 63.1, 54.4, 53.0, 18.2; HR-ESI-MS (m/z): $[M+Na]^+$ calcd for $C_{18}H_{23}N_7O_7Na$, 472.1551; found, 472.1548.

4.8. 2,3,4,6-Tetra-*O*-acetyl-β-D-galctopyranosyl-(1→4)-6-*O*-benzoyl-2,3-dibenzyl-α-D-galactopyranosyl Trichloroacetimidate (19)

CAN (0.6 g) was added at −10 °C to a cooled solution of **8** (0.5 g, 0.55 mmol) in CH_3CN (15 mL) and water (3 mL). After stirring at the same temperature for 2 h, the reaction mixture was neutralized with aq. $NaHCO_3$ and extracted with EtOAc (50 mL). Separated organic layer was dried over Na_2SO_4, filtered and chromatographed.

DBU (24 μL, 0.14 mmol) and CCl_3CN (0.29 mL, 5.8 mmol) were added at −5 °C to a cooled solution of hemiacetal in CH_2Cl_2 (8 mL). After 1 h, solvents were removed under reduced pressure and chromatographed on silica gel (30% ethyl acetate /petroleum ether) to give the desired imidate **19** as a foam (0.39 g, 76% over 2 steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (s, 1H, NH), 7.95 (d, J = 7.2 Hz, 2H, ArH), 7.56–7.52 (m, 1H, ArH), 7.43–7.27 (m, 12H, ArH), 6.51 (d, J = 3.0 Hz, 1H, H-1), 5.36 (d, J = 3.2 Hz, 1H, H-4′), 5.19 (ap.t, J = 10.0, 7.8 Hz, 1H, H-2′), 5.01 (dd, J = 10.0, 3.2 Hz, 1H, H-3′), 4.83–4.59 (m, 6H), 4.41–4.37 (m, 1H), 4.30–4.27 (m, 1H), 4.16 (d, J = 1.3 Hz, 1H, H-4), 4.07–3.97 (m, 4H), 3.82 (t, J = 6.4 Hz, IH), 2.14 (s, 3H, CH_3), 1.997 (s, 3H, CH_3), 1.995 (s, 3H, CH_3), 1.83 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.5, 170.4, 169.9, 166.4, 161.1, 138.3, 138.2, 133.2, 130.1, 129.8, 128.7, 128.6, 128.5, 128.3, 128.1, 128.04, 128.0, 127.8, 127.5, 102.3, 94.8, 91.4, 76.3, 76.0, 73.9, 73.3, 71.2, 70.9, 70.6, 69.1, 67.0, 64.7, 61.6, 20.9, 20.8, 20.8.

4.9. *N*-(Benzyloxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galctopyranosyl-(1→4)-6-*O*-benzoyl-2,3-dibenzyl-α-D-galactopyranosyl-(1→3)-2,4-diazido-2,4,6-trideoxy-α-D-glucopyranosyl)-L-serine methylester (21)

AgOTf (0.18 g, 0.7 mmol) was added to a premixed solution of glycosyl chloride **20**[14b] (0.28 g, 0.35 mmol), acceptor **18** (0.13 g, 0.29 mmol), sym. collidene (45 μL, 0.32 mmol) and 3 Å MS in CH_2Cl_2 (8 mL) at −30 °C and the reaction mixture was stirred at the same temperature for 3 h. The reaction mixture was quenched with Et_3N and the mixture was filtered through celite. Filtrate was concentrated in vacuo and chromatographed on silica gel (35% ethyl acetate/pet ether) to obtain **21** as a foam (0.29 g, 82%): $[\alpha]^{20}_D$ +54.2 (c 0.55, $CHCl_3$); IR ($CHCl_3$) ν 3020, 2928, 2108, 1748, 1216, 1052, 669 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 7.2 Hz, 2H, ArH), 7.55 (t, J = 7.2 Hz, 1H, ArH), 7.46–7.28 (m, 17H, ArH), 5.72 (d, J = 8.0 Hz, 1H, NH), 5.33–5.29 (m, 2H, H-1 & H-4″), 5.19 (ap.t, J = 10.0, 7.8 Hz, 1H, H-2″), 5.12 (s, 2H, CH_2 of Cbz), 4.97 (dd, J = 10.0, 3.4 Hz, 1H, H-3″), 4.83–4.77 (m, 3H), 4.76 (d, $J_{1',2'}$ = 3.7 Hz, IH, H-1′), 4.73–4.63 (m, 3H), 4.49–4.47 (m, 1H, CH), 4.40–4.32 (m, 2H), 4.16 (d, J = 1.6 Hz, 1H), 4.01–3.86 (m, 5H), 3.77–3.72 (m, 3H), 3.70 (s, 3H, CH_3), 3.67–3.37 (m, 1H), 3.09–3.00 (m, 2H, H-2 & H-4), 2.11 (s, 3H, CH_3), 1.97 (s, 3H, CH_3), 1.92 (s, 3H, CH_3), 1.76 (s, 3H, CH_3), 1.24 (d, J = 6.4 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.46, 170.4, 170.3, 170.1, 169.7, 166.3, 155.9, 138.5, 138.4, 136.2, 133.1, 130.3, 129.7, 128.66, 128.61, 128.5, 128.48, 128.3, 128.2, 128.0, 127.8, 127.7, 127.6, 102.0, 99.2, 99.0, 78.0, 76.1, 75.8, 75.5, 73.9, 73.6, 70.9, 70.4, 69.2, 69.0, 68.9, 68.7, 67.2, 67.1, 66.8, 64.0, 61.9, 61.2, 54.3, 53.0, 20.8, 20.7, 20.6, 18.2; HR-ESI-MS (m/z): $[M+Na]^+$ calcd for $C_{59}H_{67}O_{22}N_7Na$, 1248.4231; found, 1248.4244.

4.10. *N*-(Benzyloxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galctopyranosyl-(1→4)-6-*O*-benzoyl-2,3-dibenzyl-α-D-galactopyranosyl-(1→3)-2,4-diacetimido-2,4,6-trideoxy-α-D-glucopyranosyl)-L-serine methylester (22)

Pyridine (0.15 mL, 1.9 mmol) and water (35 μL, 1.9 mmol) were added to a clear solution of trisaccharide **21** (0.23 g, 0.19 mmol) and PPh_3 (0.2 g, 0.77 mmol) in THF (4 mL) and then the reaction mixture was kept for reflux for 4 h at 70 °C. Then solvents were removed in vacuo and the crude product was dissolved in pyridine (3 mL) and Ac_2O (0.36 mL, 3.8 mmol) was added. After stirring the reaction mixture at rt for 10 h solvents were removed under reduced pressure and the crude product was chromatographed on silica gel (60% ethyl acetate/pet ether) to obtain **22** as a foam (0.175 g, 72%): $[\alpha]^{20}_D$ +29.1 (c 1.0, $CHCl_3$); IR ($CHCl_3$) ν 3210, 3020, 2923, 1747, 1679, 1517, 1371, 1216, 1056 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 7.4 Hz, 2H, ArH), 7.67–7.28 (m, 18H, ArH), 6.21 (d, J = 8.4 Hz, 1H, NH), 5.89 (d, J = 9.4 Hz, 1H, NH), 5.81 (d, J = 7.0 Hz, 1H, NH), 5.29 (d, J = 3.0 Hz, 1H, H-4″), 5.17 (ap.t, J = 10.0, 7.8 Hz, IH, H-2″), 5.11–5.07 (m, 2H, CH_2 of Cbz), 5.00 (d, J = 2.0 Hz, 1H, H-1), 4.95 (dd, J = 10.0, 3.4 Hz, 1H, H-3″), 4.78–4.54 (m, 9H), 4.29–4.13 (m, 2H), 4.06–3.93 (m, 3H), 3.87–3.78 (m, 5H), 3.74 (s, 3H, CH_3), 3.69–3.66 (m, 2H), 3.54 (t, J = 10.0 Hz, 1H,), 2.09 (s, 3H, CH_3), 1.97 (s, 3H, CH_3), 1.87 (s, 3H, CH_3), 1.83 (s, 3H, CH_3), 1.79 (s, 3H, CH_3), 1.60 (s, 3H, CH_3), 1.10 (d, J = 6.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 170.6, 170.4, 170.3, 170.1, 169.9, 166.6, 156.2, 138.0, 136.2, 133.4, 132.2, 132.16, 132.10, 129.9, 128.9, 128.7, 128.64, 128.60, 128.5, 128.2, 128.1, 102.3, 98.9, 78.3, 75.9, 74.5, 73.7, 70.9, 70.3, 69.3, 68.8, 67.9, 67.2, 66.7, 62.7, 60.9, 67.6, 54.5, 53.0, 52.8, 23.3, 23.2, 21.0, 20.7, 20.6, 1801; HR-ESI-MS (m/z): $[M+Na]^+$ calcd for $C_{63}H_{75}O_{24}N_3Na$, 1280.4633; found, 1280.4670.

4.11. *N*-(Benzyloxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galctopyranosyl-(1→4)-6-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,4-diacetimido-2,4,6-trideoxy-α-D-glucopyranosyl)-L-serine methylester (23)

A solution of $NaBrO_3$ (0.07 g, 0.47 mmol) in water (1.5 mL) was added to a clear solution of **22** (0.1 g, 0.08 mmol) in EtOAc (1.1 mL). To this biphasic layer a solution of $Na_2S_2O_4$ (0.07 g, 0.0.4 mmol) in water (2 mL) was added dropwise over 5 min. After 45 min. the reaction mixture was quenched with aq $Na_2S_2O_3$ solution and extracted with EtOAc (30 mL × 3). Combined organic layers were dried over Na_2SO_4, concentrated and chromatographed on silica gel (5% methanol/ethyl acetate) to afford the desired product **23** as a white solid (72 mg, 85%): $[\alpha]^{20}_D$ +51.3 (c 0.59, MeOH); IR (MeOH) ν 3409, 2946, 2833, 2518, 2043, 1740, 1663, 1450, 1030 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 8.05 (d, J = 7.4 Hz, 2H, ArH), 7.62 (t, J = 7.4 Hz, 1H, ArH), 7.50 (t, J = 7.4 Hz, 2H, ArH), 7.36–7.27 (m, 5H, ArH), 5.26 (d, J = 2.6 Hz, 1H), 5.10–5.06 (m, 4H), 5.00 (d, J = 3.8 Hz, 1H), 4.67–4.61 (m, 4H), 4.45 (t, J = 4.0 Hz, 1H), 4.19–4.06 (m, 4H), 3.94–3.74 (m, 7H), 3.70 (s, 3H, CH_3), 3.58 (dd, J = 10.0, 3.8 Hz, 1H), 3.49–3.46 (m, 1H), 2.09 (s, 3H, CH_3), 2.89 (s, 3H, CH_3), 1.97 (s, 3H, CH_3), 1.92 (s, 3H, CH_3), 1.81 (s, 3H, CH_3), 1.78 (s, 3H, CH_3), 1.13 (d, J = 6.4 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 174.3, 173.6, 172.4, 172.3, 172.2, 171.8, 171.6, 167.5, 158.6, 137.9, 134.7, 131.1, 130.8, 129.9, 129.6, 129.3, 129.2, 103.3, 102.2, 99.9, 78.0, 77.6, 72.3, 71.3, 70.8, 70.3, 69.4, 69.0, 68.5, 68.3, 68.1, 63.4, 62.1, 58.5, 56.0, 54.5, 53.1, 23.2, 23.0, 21.2, 20.6, 20.4, 18.1; HR-ESI-MS (m/z): $[M+H]^+$ calcd for $C_{49}H_{64}O_{24}N_3Na$, 1078.3879; found, 1078.3871.

4.12. *N*-(Benzyloxycarbonyl)-*O*-(β-D-galctopyranosyl-(1→4)-α-D-galactopyranosyl-(1→3)-2,4-diacetimido-2,4,6-trideoxy-α-D-glucopyranosyl)-L-serine (24)

A solution of NaOMe (35 mg) in MeOH (2 mL) was added to a clear solution of **23** (33 mg, 0.03 mmol) in MeOH (2 mL) and water (2 mL) at 50 °C (pH 10). After stirring the reaction mixture at the same temperature for 20 h, the reaction mixture was neutralized with AcOH until the pH adjusted to 6. Then solvents were removed under reduced pressure and the crude product was chromatographed on silica gel (7:2:1 ethyl acetate/MeOH/H_2O) to afford the desired product **24** as a white solid (22 mg, 89%): $[\alpha]^{20}_D$ +43.8 (*c* 0.32, MeOH); IR (MeOH) *ν* 3544, 2831, 2522, 1661, 1449, 1415, 1032, 688 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) *δ* 7.38–7.29 (m, 5H, ArH), 5.10 (d, *J* = 6.5 Hz, 2H), 4.97 (d, *J* = 2.8 Hz, 1H), 4.65–4.61 (m, 1H), 4.42 (d, *J* = 8.0 Hz, 1H), 4.22–4.16 (m, 1H), 4.06–4.03 (m, 2H), 3.88–3.33 (m, 16H), 2.03, 1.96 (2s, 3H, CH_3), 1.89 (s, 3H, CH_3), 1.05 (d, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, CD_3OD) *δ* 180.3, 176.8, 174.1, 158.0, 129.6, 129.2, 129.0, 107.1, 102.2, 101.9, 99.4, 98.9, 80.5, 79.5, 78.2, 77.6, 77.1, 75.3, 73.4, 71.9, 71.6, 71.0, 70.5, 70.2, 68.3, 68.0, 67.8, 62.6, 61.1, 60.9, 58.2, 58.1, 57.7, 54.7, 24.2, 23.2, 18.2; HR-ESI-MS (*m*/*z*): $[M+Na]^+$ calcd for $C_{33}H_{48}O_{19}N_3Na_2$, 836.2672; found, 836.2675.

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