Tetrahedron Letters 52 (2011) 3320–3323

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

A convenient synthesis of the core trisaccharide of the *N*-glycans

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ARTICLE INFO

Article history:
Received 15 February 2011
Revised 6 April 2011
Accepted 15 April 2011
Available online 23 April 2011

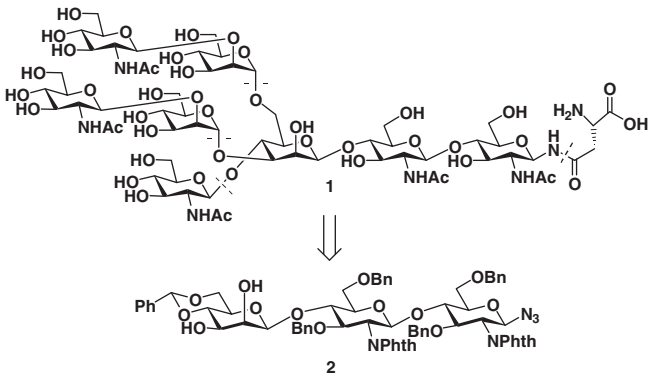
Keywords:
N-Glycan
One-pot glycosylation
Core trisaccharide
1,2-Orthoester

ABSTRACT

A convenient synthesis of the core trisaccharide of the *N*-glycans was described. Orthogonal one-pot glycosylation of three monosaccharide building blocks was performed to furnish β-glucosyl chitobiose, which was then transformed to β-mannosyl chitobiose by intramolecular epimerization of the C-2 position of the β-glucoside. The key glucosyl donor **7c** with differentiated 2,3-OH was prepared following the 4,6-*O*-benzylidene-protected 1,2-orthoester strategy.

Glycoproteins with *N*-glycans found on cell surfaces and in the blood serum play important roles in many biological events such as cell–cell adhesion, immune system modulation, and signal transduction. Subtle changes of the carbohydrate moieties may result in completely altered functionality of the glycoproteins. Therefore, asparagine linked *N*-glycans have gained intensive investigations.[1] Actually, only a few of these oligosaccharides can be isolated from natural sources due to their microheterogeneity.[2] These circumstances have stimulated the chemical synthesis of *N*-glycans as a method to provide sufficient quantities for further research.[3–9] All types of *N*-glycans comprise a common core structure, the β-1,4-linked D-mannosyl chitobiose unit, suggesting that a modular approach based on this scaffold could be particularly useful.[10] An efficient assembly of this appropriately protected core structure will facilitate the development of practical synthesis of N-linked glycoproteins. (Scheme 1) Thus, a great deal of effort has been devoted to the synthesis of this core trisaccharide[11–14]

One part of the work for the synthesis of the core trisaccharide is the daunting construction of β-mannosyl glycosidic bond. Several methods have been developed for β-mannoside synthesis. These include direct β-D-mannoside coupling methods,[15] the intramolecular aglycon delivery strategy,[16] sequential oxidation/reduction routes[17] as well as epimerization of β-D-glucopyranosides methods,[12,18] all of which have their respective advantages and shortcomings. Owing to usually excellent yield and complete stereoselectivity, intramolecular epimerization of β-glucosides to β-mannosides can be regarded as a reasonably safe way for the construction of β-mannosides.[13] Based on this method and 1,2-orthoester strategy, we prepared the core trisaccharide **2** for the synthesis of *N*-glycan octasaccharide of the bisecting type (Scheme 1) in our previous work.[14] The key intermediate orthoester **B** (Scheme 2) was designed for the construction of the trisaccharide **2** and the following assembly of octasaccharide **1**. The 1,2-orthoester unit allowed the differentiation of 2,3-OH in β-D-glucopyranosyl moiety. While the cyclic 4,6-*O*-benzylidene permitted the differentiation of OH-3 from OH-4 and OH-6, which facilitated the introduction of three antennas. However, this approach still needs to be improved in two aspects. Firstly, step by step glycosylation for the trisaccharide construction resulted in multistep protecting manipulation and intermediate purification. Secondly, since thioglycoside **G** and azide acceptor **F** were valuable



Scheme 1. Chemical structures of octasaccharide-asparagine **1** and core trisaccharide **2**.

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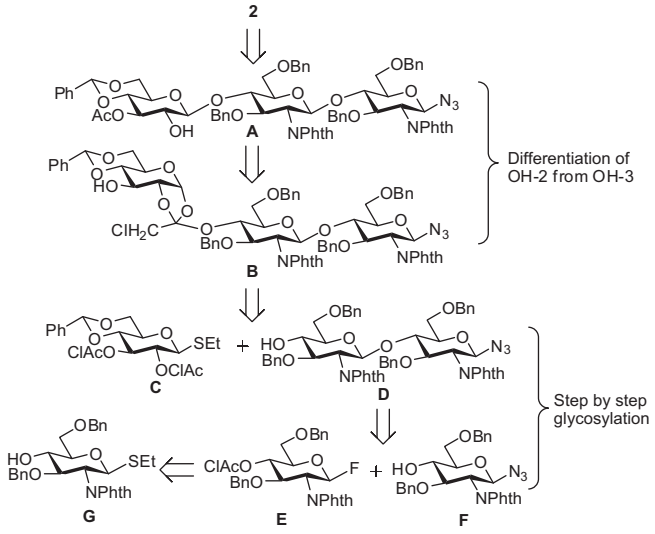
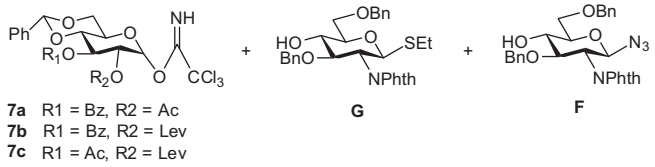
doi:10.1016/j.tetlet.2011.04.060



Scheme 2. Previous synthesis of the core trisaccharide **2**.



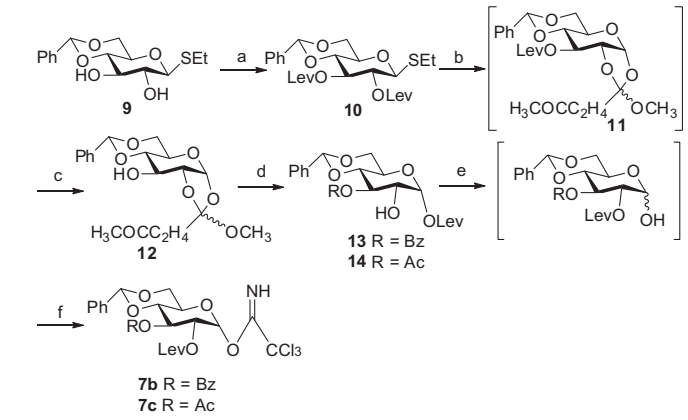
Scheme 3. Three building blocks for one-pot glycosylation.

building blocks, it's not economical to differentiate 2,3-diols of the glucosyl residue after the construction of the trisaccharide.

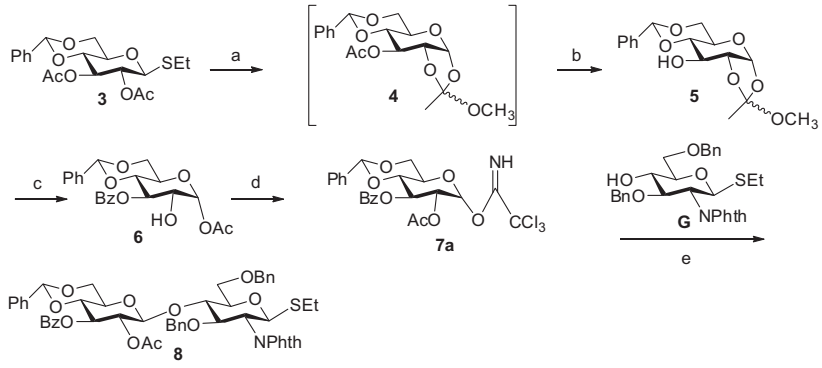
We herein describe an improved approach to the synthesis of the core trisaccharide **2**. The key glucosyl trichloroacetimidate **7**[19] with well differentiated 2,3-diols, the thioglycoside **G**[20], and azide acceptor **F**[12] were selected for orthogonal one-pot glycosylation. (Scheme 3) Then the β-mannosyl chitobiose was established by intramolecular epimerization of the C-2 position of the β-glucoside. Compared with the former described differentiation of the 2,3-diols after the glycosylation, this prior differentiation in the glucosyl donor stage diminished the loss of valuable building blocks **F** and **G**. In addition, the one pot glycosylation simplified the procedure for construction of the β-glucosyl chitobiose.

The 4,6-*O*-benzylidene-protected 1,2-orthoester, which has been investigated by our group, can be prepared effectively and applied for differentiation of 2,3-OH of monoglucopyranose.[19] Based on this approach, the trichloroacetimidate **7a** with the 2,3-OH masked by two orthogonal groups was synthesized efficiently from orthoester **5.**[19] On account of the difficult removal of the sterically hindered 2-acetate,[12] disaccharide **8** was first synthesized and then applied for the subsequent selective removal of the acetyl group (Scheme 4) before the one-pot glycosylation. However, the compatibility of protecting groups in compound **8** was not satisfactory, since many reaction conditions for subsequent selective removal of acetyl group were tested but failed. For instance, treating compound **8** with acetyl chloride/methanol led to the deprotection of 4,6-*O*-benzylidene, while switched to typical basic conditions (DBU, K_2CO_3, $NH_2NH_2{\cdot}H_2O$, $Mg(OEt)_2$), the cleavage of benzoyl group was observed.

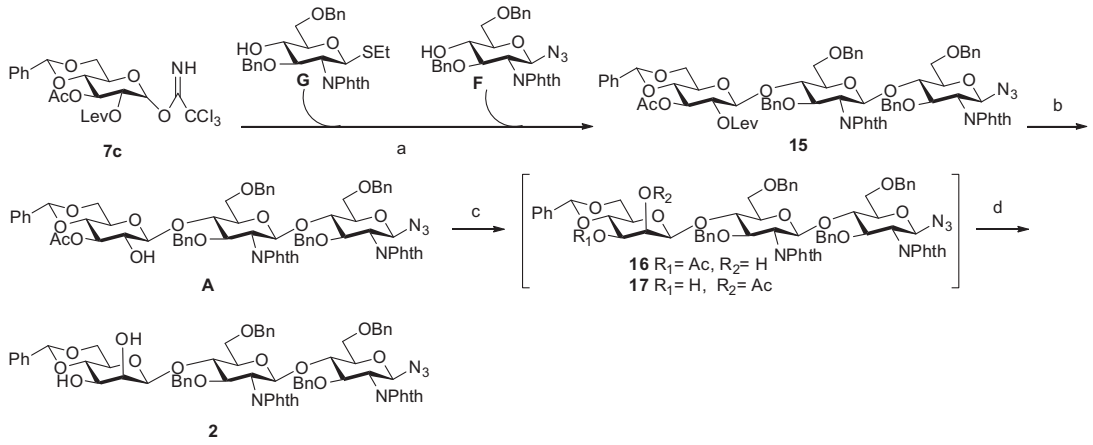
To circumvent this difficulty, new designed glucosyl donors **7b/7c** were prepared following a similar method to the preparation of **7a** (Scheme 5).[19] The difference is that these two donors were prepared from a new type of orthoester **12**. In addition, it was equipped with an easier removable levulinyl group (Lev) in C-2 position. The synthesis started from compound **9**,[21] which was converted to compound **10** with 2,3-diols protected by Lev in 95% yield. When activated by *N*-bromosuccinimide (NBS)/TMSOTf in the presence of CH_3OH and subsequently cleaved Lev in one pot, compound **10** was transformed to orthoester **12**[24] with two iso-



Scheme 5. Reagents and conditions: (a): LevOH, EDC·HCl, DMAP, DCM, 2 h, 42 °C, 95%; (b): NBS, TMSOTf, DCM:CH_3OH = 50:1, 4 Å MS, 0 °C, 0.5 h, (c) CH_3ONa, CH_3OH, 83% for two steps; (d): for compound **13**: (1) BzCl, pyridine, DCM, DMAP; (2) 1 mol/L hydrochloric acid, Silica, 81%; for compound **14**: (1) Ac_2O, Pyridine, DCM, DMAP; (2) 1 mol/L hydrochloric acid, Silica, 87%; (e): Et_3N/DCM = 1:4; 12 h. (f): DBU, CCl_3CN, DCM. Compound **7b** (86%) or **7c** (91%) for two steps.



Scheme 4. Reagents and conditions: (a): NBS, TMSOTf, DCM:CH_3OH = 50:1, 4 Å MS, 0 °C, 0.5 h, (b) CH_3ONa, CH_3OH, 89% for two steps; (c): (1) BzCl, pyridine, DCM, DMAP; (2) 2 mol/L hydrochloric acid, Silica, 81% for two steps; (d): (1) Et_3N, DCM; 12 h; (2) DBU, CCl_3CN, DCM, 88% for two steps; (e): TMSOTf, DCM, 4 Å molecular sieve (MS), −15 °C, 0.5 h, 65%.



Scheme 6. Reagents and conditions: (a): (1) **G**, TfOH, DCM, 4 Å MS, −15 °C, 0.5 h; (2) **F**, NIS, 4 Å MS, −15 °C, 0.5 h, 81%; (b): N_2H_4·HOAc, DCM:CH_3OH = 1:1, rt, 1 h, 89%; (c): (1) Tf_2O, pyridine, −15 °C, 3 h; 2) DMF:H_2O = 20:1, 50 °C, 24 h; (d) CH_3ONa, CH_3OH, 87%.

mers (1:2) in 83% yield. After acetylation or benzoylation of OH-3 the hydrolysis of 1,2-orthoester intermediate with 1 M hydrochloric acid afforded compound **13** (81%) or **14** (87%). Notably, the hydrolysis procedure could be accelerated by the addition of silica gel. Then the anomeric center was liberated with the influence of Et_3N in CH_2Cl_2 and the resulting intermediate was converted to the trichloroacetimidate donor **7b** (86%) or **7c** (91%) according to the method developed by Schmidt.[22]

With donor **7b** and **7c** in hand, the glycosylation with acceptor **G** was conducted firstly. Under a similar condition for the glycosylation of **7a** with **G**, donor **7b** and **7c** provided the corresponding disaccharides in yields of 78% and 85%, respectively. Due to better yield for donor **7c**,[25] it was selected for the following orthogonal one-pot glycosylation.[23] As shown in Scheme 6, trichloroacetimidate donor 7**c** (1.3 equiv) was activated by TfOH at −15 °C to be coupled with acceptor **G** (1.0 equiv). When TLC showed the full consumption of **G** (0.5 h), NIS and acceptor **F** (1.0 equiv) were added. The mixture was stirred for another 0.5 h, giving the resulting trisaccharide **15** in 81% yield.[26] Subsequent selective cleavage of Lev by N_2H_4·HOAc was conducted smoothly to afford intermediate **A** in high yield (89%). The transformation of β-glucoside to β-mannoside was performed through the intramolecular inversion of the C-2 hydroxyl of **A** by a three-step procedure with excellent reproducibility and complete stereoselectivity.[14] Firstly, the OH-2 position was activated with trifluoromethylsulfonic anhydride and pyridine. The intermediate triflate was substituted by an intramolecular nucleophilic attack of the neighboring acetyl moiety to afford a mixture of compound **16** and isomer **17**. Subsequent deprotection of the acetyl group by methanolysis gave the desired core trisaccharide **2** in 87% yield over the reaction sequence. It should be noted that no further purifications were needed after simple workup for the intermediates during this three-step procedure to convert **A** to compound **2.**[27]

In conclusion, we have presented a convenient synthesis of the core trisaccharidic scaffold for the assembly of *N*-glycans. Our route improves the published procedure in simplicity and yield. Notably, three monosaccharide building blocks for one-pot glycosylation were easily prepared. Owing to its facility and mild reaction conditions, the procedure can be applied to access a series of *N*-glycans.

Acknowledgments

This work is supported by the National Nature Science Foundation of China (NSFC21002014) and the Fundamental Research Funds for the Central Universities of China.

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19. *General procedure for the preparation of compound* **5**. (The procedure for the preparation of **12** is same as the compound **5**): After a mixture of compound **3** (1.0 mmol), CH_3OH (1 mL) and 4 Å molecular sieves in freshly distilled CH_2Cl_2 (50 mL) were stirred for 0.5 h at rt and then cooled to 0 °C, NBS (178 mg, 1.0 mmol) was added and the reaction mixture was stirred for 2 min. TMSOTf (18 μL, 0.1 mmol) was added dropwise and the reaction mixture was stirred for 15 min at 0 °C. Then NBS (178 mg, 1.0 mmol) and TMSOTf (18 μL, 0.1 mmol) were added for the second time. The reaction mixture was stirred for another 15 min at 0 °C, TLC (*n*-Hexane/EtOAc = 2:1) indicated that compound **3** completely converted to intermediate 1,2-orthoesters **4**. Then a solution of CH_3ONa in MeOH (0.1 M, 40 mL) was added dropwise to the reaction mixture. The solution was allowed to warm to rt and stirred for 30 min, then filtered through Celite. Silica gel (activated by Et_3N, 4 g) was added to the filtrate, and then concentrated in vacuo. The crude mixture was purified by column chromatography on Silica gel (Silica gel had been activated by Et_3N) to afford orthoester **5**.
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24. *Physical data for compound* **12**: ^{1}H NMR: (600 MHz, $CDCl_3$): (mixture of isomers, the ratio is 1:2) δ 7.53–7.45 (m, 3H), 7.40–7.32 (m, 4.5H), 5.75 (d, *J* = 5.5 Hz, 1H), 5.59 (d, *J* = 5.8 Hz, 0.5H), 5.53 (d, 1.5H), 4.36 (ddd, *J* = 10.6, 5.3, 1.4 Hz, 1.5H), 4.31–4.23 (m, 1.5H), 4.14 (t, *J* = 5.7 Hz, 0.5H), 3.99 (dd, *J* = 9.5, 5.4 Hz, 1H), 3.89 (m, 0.5H), 3.78 (td, *J* = 9.8, 5.2 Hz, 1H), 3.68 (t, *J* = 10.3 Hz, 1.5H), 3.50 (td, *J* = 9.6, 7.8 Hz, 1.5H), 3.36 (s, 1.5H), 3.24 (s, 3H), 2.68–2.58 (m, 2H), 2.58–2.53 (m, 1H), 2.20 (m, 2H), 2.15 (s, 3H), 2.14 (s, 1.5H), 2.14–2.10 (m, 1H). ^{13}C NMR: (150 MHz, $CDCl_3$): δ 207.9, 207.7, 177.5, 136.8, 136.8, 129.3, 128.3, 126.2, 126.1, 121.7, 121.0, 101.8, 101.6, 99.0, 98.4, 79.1, 78.9, 78.3, 73.3, 73.1, 68.5, 68.5, 63.1, 62.9, 60.4, 50.8, 49.7, 45.7, 38.0, 37.9, 30.5, 30.1, 29.5, 28.7, 14.1. HRMS (ESI): *m*/*z* calcd for $C_{19}H_{24}O_8Na$: $[M+Na]^+$ 403.1369, found 403.1359.
25. *Physical data for compound* **7c**: $[\alpha]_D^{23}$ +49.6 (*c* 0.1, $CHCl_3$); ^{1}H NMR: (400 MHz, $CDCl_3$): δ 8.67 (s, 1H), 7.44 (m, 2H), 7.35 (m, 3H), 6.52 (d, 1H, *J* = 3.5 Hz), 5.69 (t, 1H, *J* = 9.8 Hz), 5.54 (s, 1H), 5.17 (dd, 1H, *J* = 10.0, 3.7 Hz), 4.35 (dd, 1H, *J* = 10.4, 4.9 Hz), 4.16–4.10 (m, 1H), 3.81–3.74 (m, 1H), 2.73–2.70 (m, 2H), 2.54–2.50 (m, 2H), 2.16 (s, 3H), 2.12 (s, 3H). ^{13}C NMR: (100 MHz, $CDCl_3$): δ 205.9, 171.6, 169.9, 161.0, 136.6, 129.1, 128.3, 126.1, 101.6, 93.5, 90.8, 78.6, 70.5, 68.5, 68.5, 65.1, 37.5, 29.7, 27.7, 20.8.
26. *General procedure for the one-pot synthesis of trisaccharide* **15**: after a mixture of **7c** (75 mg, 0.14 mmol), compound **G** (56 mg, 0.11 mmol) and pre-activated 4Å molecular sieves in freshly distilled CH_2Cl_2 (10 mL) was stirred at rt for 0.5 h and then cooled to −15 °C, TfOH (2.8 μL, 0.03 mmol) was added dropwise. The mixture was stirred for 0.5 h at −15 °C. Then compound **F** (54 mg, 0.11 mmol) and NIS (31 mg, 0.14 mmol) was added. The mixture was stirred for 30 min at −15 °C and then filtered through Celite. The filtrate was diluted with CH_2Cl_2 and then washed with aqueous $NaHCO_3$, aqueous $Na_2S_2O_3$, and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude mixture was purified by column chromatography on silica gel (EtOAc/petroleum ether = 1:3→1:2) to afford the trisaccharide **15** (117 mg, 81%) as a light yellow foam. $[\alpha]_D^{23}$ +3.1 (*c* 0.1, $CHCl_3$); ^{1}H NMR: (400 MHz, $CDCl_3$): δ 7.85 (d, 1H, *J* = 7.4 Hz), 7.80–7.62 (m, 6H), 7.54 (s, 1H), 7.47–7.30 (m, 15H), 7.00 (d, 2H, *J* = 6.3 Hz), 6.96–6.88 (m, 5H), 6.77–6.76 (m, 3H), 5.37 (s, 1H), 5.29 (d, 1H, *J* = 8.2 Hz), 5.21 (t like, 1H, *J* = 9.4, 9.8 Hz), 5.15 (d, 1H, *J* = 9.4 Hz), 4.97 (t like, 1H, *J* = 7.8, 9.4 Hz), 4.89 (d, 1H, *J* = 12.5 Hz), 4.78 (d, 1H, *J* = 12.1 Hz), 4.72 (d, 1H, *J* = 7.8 Hz), 4.65 (d, 1H, *J* = 12.1 Hz), 4.58–4.37 (m, 5H), 4.31–4.11 (m, 8H), 4.04 (t, 1H, *J* = 9.8 Hz), 3.75–3.68 (m, 2H), 3.56–3.49 (m, 2H), 3.44–3.38 (m, 3H), 3.25 (m, 1H), 2.79–2.64 (m, 2H), 2.55–2.35 (m, 2H), 2.17 (s, 3H), 2.07 (s, 3H). ^{13}C NMR: (100 MHz, $CDCl_3$): δ 205.6, 171.4, 170.2, 138.6, 138.5, 138.2, 137.8, 136.8, 133.8, 131.5, 129.1, 128.6, 128.2, 128.0, 127.9, 127.9, 127.8, 127.7, 127.6, 127.4, 127.1, 126.9, 123.3, 101.4, 100.5, 97.0, 85.5, 78.5, 78.0, 75.4, 74.7, 74.6, 73.3, 72.7, 71.6, 68.4, 67.6, 66.9, 66.1, 56.5, 55.2, 37.6, 29.7, 27.8, 20.8. HRMS (ESI): *m*/*z* calcd for $C_{76}H_{73}N_5O_{20}Na$: $[M+Na]^+$ 1398.4741, found 1398.4798.
27. *Physical data for compound* **2**: $[\alpha]_D^{23}$ +11.6 (*c* 0.1, $CHCl_3$) ^{1}H NMR: (400 MHz, $CDCl_3$): δ 7.86(s, 1H), 7.71–7.67 (m, 6H), 7.56 (s, 1H), 7.46–7.44 (m, 2H), 7.38–7.28 (m, 14H), 7.02 (d, 2H, *J* = 7.0 Hz), 6.97–6.90 (m, 5H), 6.81–6.75 (m, 3H), 5.45 (s, 1H), 5.30 (d, 1H, *J* = 8.6 Hz), 5.17 (d, 1H, *J* = 9.4 Hz), 4.84 (t like, 2H, *J* = 12.1, 11.4 Hz), 4.75 (s, 1H), 4.59 (t like, 2H, *J* = 12.9, 12.1 Hz), 4.53–4.49 (m, 3H), 4.46–4.38 (m, 2H), 4.29 (t like, 1H, *J* = 9.4, 9.0 Hz), 4.23(dd, 1H, *J* = 10.6, 8.6 Hz), 4.17–4.12 (m, 3H), 4.06 (t like, 1H, *J* = 10.6, 9.4 Hz), 3.94 (d, 1H, *J* = 3.1 Hz), 3.76 (t, 1H, *J* = 9.4 Hz), 3.67 (d, 1H, *J* = 11.7 Hz), 3.62–3.57 (m, 3H), 3.54 (t like, 1H, *J* = 10.6, 10.2 Hz), 3.44 (dd, 1H, *J* = 11.4, 3.5 Hz), 3.40–3.38 (m, 1H), 3.34–3.32 (m, 1H), 3.15–3.11 (m, 1H). ^{13}C NMR: (100 MHz, $CDCl_3$): δ 138.3, 138.1, 137.5, 137.1, 133.8, 131.5, 129.2, 128.6, 128.3, 128.3, 128.0, 128.0, 127.9, 127.9, 127.5, 127.3, 127.2, 127.0, 126.2, 123.7, 123.4, 102.0, 100.4, 96.9, 85.6, 78.7, 78.4, 77.6, 76.7, 76.3, 75.0, 74.7, 74.5, 74.4, 73.5, 72.7, 70.7, 68.4, 67.7, 66.6, 56.5, 55.2. HRMS (ESI): *m*/*z* calcd for $C_{69}H_{65}N_5O_{17}Na$: $[M+Na]^+$ 1358.4273, found 1358.4268.