Successful Combination of (Methoxydimethyl)methyl (MIP) and (2-Naphthyl)methyl (NAP) Ethers for the Synthesis of Arabinogalactan-Type Oligosaccharides

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Abstract: In order to characterise the presumed epitopes of arabinogalactans, isolated from the extract of the cell-cultured *Echinacea purpurea*, two oligosaccharides were synthesized. The whole synthetic route was based on the successful combination of the (methoxydimethyl)methyl (MIP) and the (2-naphthyl)methyl ether (NAP) protecting groups. A β-(1→6)-linked trigalactoside was prepared, which contained a NAP ether at position 2′.This protecting group was selectively removed using either DDQ or Pd-C/H_2 and the acceptor was ready for further glycosylation.We have used two arabinofuranosyl donor compounds: 2,3,5-tri-*O*-acetyl arabinofuranosyl trichloroacetimidate and a peracetylated α-(1→5)-linked diarabinofuranosyl trichloroacetimidate. For the deprotection of the tetra- and pentasaccharides a common procedure was used. All of the synthesized compounds were characterized by ^{1}H and ^{13}C NMR spectroscopy, as well as by MALDI-TOF mass-spectrometry.

Key words: arabinogalactan, oligosaccharides, protecting groups, (methoxydimethyl)methyl ether (MIP), (2-naphthyl)methyl ether (NAP)

Monoclonal antibodies play a very important role in the structure determination of plant polysaccharides.[1] Their production or the evaluation of their specificity require well-defined oligosaccharides which are the epitopes of the antigens. Among the plant cell-wall polysaccharides or the plant cell exudates the arabinogalactans are very common representatives. Unfortunately, the number of the L-arabinose and D-galactose containing synthetic oligosaccharides is rather limited.[2–9] Study of the structure of arabinogalactans isolated from cell-cultured *Echinacea purpurea* required[10–12] the following two oligosaccharides (Figure).

The methoxycarbonyl undecyl glycoside[2] of compound **1** has been prepared recently, but its preparation needed many synthetic steps. Generally, the chemical synthesis of complex oligosaccharides is a rather laborious task and very often the combination of the available and the suitable protecting groups is the bottleneck of the whole synthesis. Here, we report on the successful combination of two relatively new protecting groups; (methoxydimethyl)methyl (MIP)[13] and (2-naphthyl)methyl (NAP) ethers.[14–16]

It was shown that treating either the β-D-galactopyranosyl-(1→6)-D-galactose, or the β-D-galactopyranosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose with 2,2-dimethoxy propane in the presence of protic or Lewis acid resulted in 3,4-*O*-isopropylidene-6-*O*-(methoxydimethyl)methyl-β-D-galactopyranosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose[3] (**3**). Compound **3** has a free OH-2′ group, but its glycosylation is hampered because during the glycosylation reaction the MIP group can either hydrolyze or can form, by condensation, a stable isopropylidene acetal. On the other hand, compound **3**, bearing the acetal-type MIP group, can be readily (2-naphthyl)methylated to give **4** in excellent yield. The MIP group can be cleaved by very mild acid hydrolysis to give compound **5** with a free 6′-OH group. **5** was subsequently glycosylated with α-acetobromo-D-galactose to furnish the fully protected trisaccharide **6**. From compound **6** the NAP group was removed either by oxidation using DDQ or by catalytic hydrogenolysis with Pd-C as catalyst, yielding the trisaccharide type aglycon (**7**) (Scheme 2).

It is worth to mention that the NAP group, like the benzyl group, can be removed by catalytic hydrogenation but the NAP is more sensitive and can be selectively deprotected in the presence of latter one.[17] The NAP group is very similar to the *p*-methoxybenzyl, but the NAP is more resistant

β-D-Galp-(1→6)-β-D-Galp-(1→6)-D-Gal (**1**)
2
↑
α-L-Araf-1

β-D-Galp-(1→6)-β-D-Galp-(1→6)-D-Gal (**2**)
2
↑
α-L-Araf-(1→5)-α-L-Araf-1

Figure

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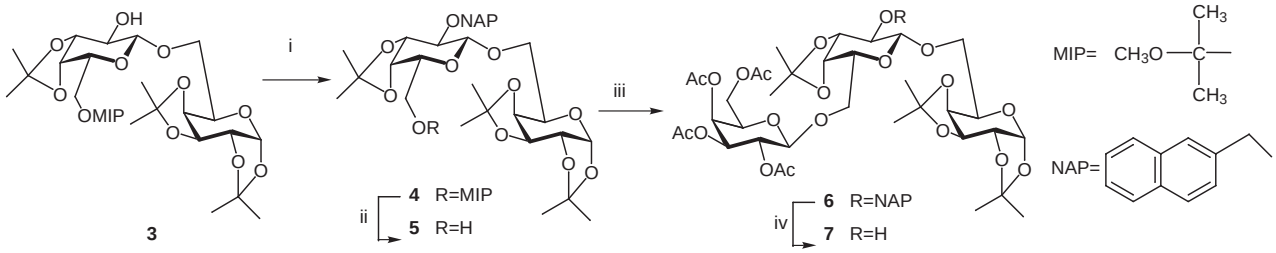
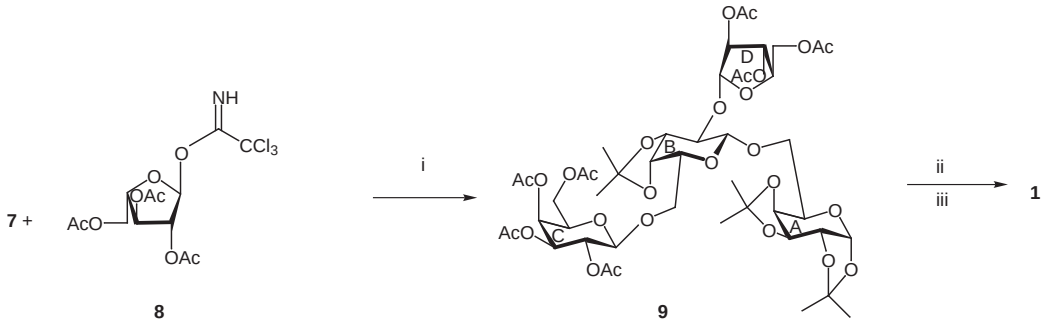


Scheme 1 i) NaH, NAPBr, DMF, 0 °C→ r.t., 78%; ii) CH_3COOH, H_2O, reflux, 3 h, 73%; iii) 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide, anhyd CH_3CN, $Hg(CN)_2$, r.t., 55% iv) CH_2Cl_2–CH_3OH, 4:1, DDQ (1.5 equiv), r.t., 89%



Scheme 2 i) anhyd CH_2Cl_2, TMSOTf, –45 °C→ r.t., 1 h, 69%; ii) NaOMe, MeOH, r.t., 4 h, quant.; iii) 90% aq. CF_3COOH, r.t., 15 min.

to acids and can survive the removing of the isopropylidene groups.[14] The *p*-methoxybenzyl derivatives are not compatible with the MIP technology.

Compound **7** was treated with 2,3,5-tri-*O*-acetyl-α,β-L-arabinofuranosyl trichloroacetimidate **8** in the presence of trimethylsilyltriflate in dichloromethane to give the fully protected tetrasaccharide **9**. Its structure was confirmed by ^{1}H- and ^{13}C NMR spectra, as well as by MALDI-TOF MS measurement (Scheme 2).

The synthetic plan for the pentasaccharide followed the route used for compound **9**, but employing a (1→5)-diarabinoside derivative as glycosyl donor. 1,2,3,5-Tetra-*O*-acetyl-α-L-arabinofuranose was treated with *p*-methoxyphenol in dichloromethane using $BF_3{\cdot}Et_2O$ as catalyst. The glycoside acetate (**11**) was deacetylated and the crystalline *p*-methoxyphenyl α-L-arabinofuranoside (**12**) was used as aglycon in an 'open glycosylation' reaction using 2,3,5-tri-*O*-acetyl-α,β-L-arabinofuranosyl trichloroacetimidate as donor and trimethylsilyltriflate as catalyst. Similar procedure was applied for the synthesis of α-L-Ara*f*-(1→5)-α-L-Ara by Kong et al.[4] using methyl α-L-arabinofuranoside as aglycon and 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl trichloroacetimidate as donor. The coupling product (**13**) was isolated, acetylated and the disaccharide derivative **14** was fully characterized by spectroscopic methods. The aglycon of compound **14** was easily removed by oxidative methods (CAN or DDQ) to obtain a free anomeric OH group (**15**), which was then transformed into trichloroacetamidate derivative (**16**) (Scheme 3).

Glycosylation of compound **7** with imidate **16** in the presence of trimethylsilyltriflate gave the fully substituted pentasaccharide **17** (Scheme 4).

For the deprotection of compounds **9** and **17** common procedures were followed; Zemplén's deacylation and consecutive mild hydrolysis of the isopropylidene groups by trifluoroacetic acid resulted in the free tetra- (**1**) and pentasaccharide (**2**).

The identity and stereochemical integrity of all compounds described here were verified by HPLC and by ^{1}H- and ^{13}C NMR spectra. MALDI-TOF MS measurements provided further support for these structures. The characteristic NMR, MALDI-TOF MS and physical data are given in refs.[18–22]

In summary, we have demonstrated the useful combination of MIP [(methoxydimethyl)methyl] and NAP [(2-naphthyl)methyl ether] groups for the preparation of branched arabinogalactan-type oligosaccharides. Presumably these oligosaccharides are epitopes of arabinogalactan polysaccharides isolated from cell-cultured exudates of *Echinacea purpurea*.

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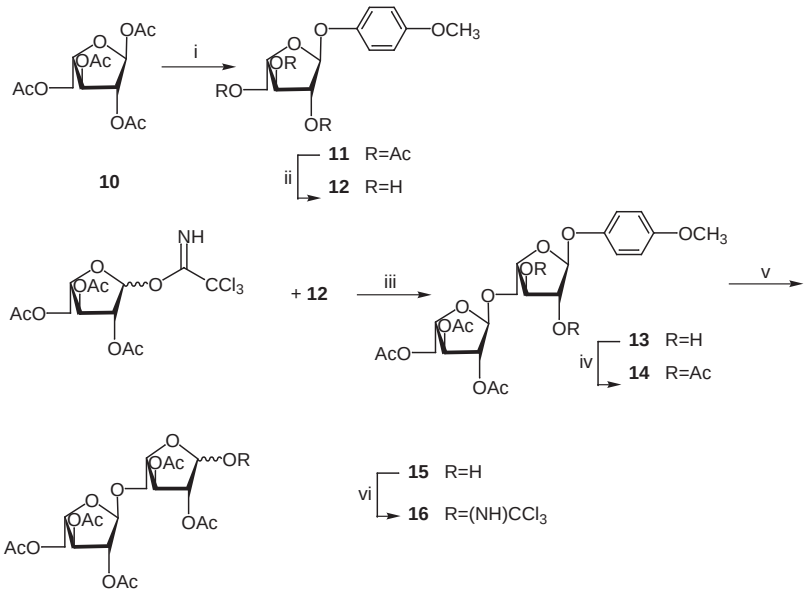
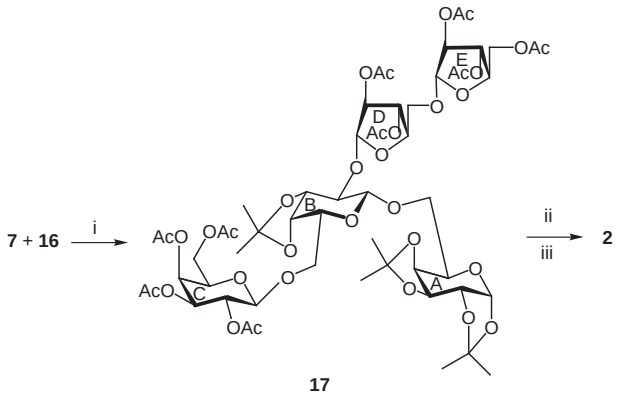


Scheme 3 i) 4-Methoxyphenol, $BF_3{\cdot}Et_2O$, 0 °C, 12 h, 77%; ii) $NaOCH_3$, MeOH, r.t., 12 h, 73%; iii) anhyd CH_2Cl_2, TMSOTf (0.15 equiv), –42 °C, 1 h, 32% iv) Ac_2O, pyridine, r.t., 2 h, 83%; v) CAN (5 equiv), toluene–CH_3CN–H_2O = 1:2:1, r.t., 1 h, 83%; vi) K_2CO_3, CCl_3CN, r.t., 12 h.



Scheme 4 i) anhyd CH_2Cl_2, TMSOTf, -45 °C→ r.t., 1 h, 65%; ii) NaOMe, MeOH, r.t., 4 h, quant.; iii) 90% aq CF_3COOH, r.t., 15 min.

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(18) Spectroscopic data of compound **9**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.44 (d, 1 H, $J_{1,2}$ = 5.6 Hz, A-1), 4.26–4.28 (m, 1 H, A-2), 4.59 (dd, 1 H, $J_{2,3}$ = 7.9 Hz, $J_{3,4}$ = 2.3 Hz, A-3), 4.19 (dd, 1 H, $J_{4,5}$ = 1.9 Hz, A-4), 3.98–4.1 (m, 1 H, A-5), 4.15 (d, 1 H, $J_{5,6}$ = 8.6 Hz, A-6a), 3.58 (dd, 1 H, $J_{6a,6b}$ = 10.3 Hz, A-6b), 4.26 (d, 1 H, $J_{1,2}$ = 5.2 Hz, B-1), 3.82 (dd, 1 H, $J_{2,3}$ = 8.3 Hz, B-2), 4.25 (s, 1 H, B-3), 4.07 (dd, 1 H, $J_{4,5}$ = 1.9 Hz, B-4), 3.94–3.96 (m, 1 H, B-5), 4.05 (dd, 1 H, $J_{5,6}$ = 2.3 Hz, $J_{6a,6a}$ = 7.7 Hz, B-6a), 3.94–3.98 (m, 1 H, B-6b), 4.72 (d, 1 H, $J_{1,2}$ = 8.1 Hz, C-1), 5.23 (dd, 1 H, $J_{2,3}$ = 10.5 Hz, C-2), 5.01 (d, 1 H, $J_{3,4}$ = 3.4 Hz, C-3), 5.38 (dd, 1 H, $J_{4,5}$ = 0.9 Hz, C-4), 3.92–3.95 (m, 1 H, C-5), 4.15 (d, 2 H, $J_{6a,6b}$ = 6.7 Hz, C-6a, C-6b), 5.45 (s, 1 H, d-1), 5.13 (s, 1 H, D-2), 4.99 (d, 1 H, $J_{3,4}$ = 3.7 Hz, D-3), 4.68 (dd, 1 H, $J_{4,5}$ = 7.1 Hz, D-4), 4.48 (dd, 1 H, $J_{5,6a}$ = 3.4 Hz, $J_{6a,6b}$ = 12.5 Hz, D-5a), 4.32 (dd, 1 H, $J_{5,6b}$ = 2.5 Hz, D-5b), 1.25, 1.28, 1.38, 1.49, 1.52 (s, 24 H, 6 CH_{3ip}), 1.95, 2.02, 2.08, 2.01, 2.13, 2.15 (s, 21 H, 7 $CH_{3acetyl}$). ^{13}C NMR (500 MHz, $CDCl_3$): 95.71 (A-1), 69.59 (A-2), 70.47 (A-3), 71.16 (A-4), 66.80 (A-5), 69.40 (A-6), 100.78 (B-1), 73.94 (B-2), 79.55 (B-3), 73.46 (B-4), 70.36 (B-5), 68.38 (B-6), 101.02 (C-1), 68.46 (C-2), 70.44 (C-3), 66.74 (C-4), 72.85 (C-5), 60.88 (C-6), 103.07 (D-1), 80.52 (D-2), 76.62 (D-3), 80.49 (D-4), 63.58 (D-5), 20.28, 20.36, 20.51, 20.54, 20.68 ($CH_{3acetyl}$), 23.75, 24.51, 25.60, 25.69, 26.02, 27.71 (CH_{3ip}), 108.03, 108.55, 110.34 (C_{quat}).

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$[\alpha]_D$: –45.32 (c 0.26, $CHCl_3$). MALDI-TOF measurements: 1073.25 $[M + Na]^+$

(19) Spectroscopic data of compound **14**: ^1H NMR (500 MHz, $CDCl_3$): δ 5.59 (s, 1 H, *H-1*), 5.32 (d, 1 H, $J_{2,3}$ = 2.16 Hz, *H-2*), 5.28 (dd, 1 H, $J_{3,4}$ = 6.5 Hz, *H-3*), 4.32–4.35 (m, 1 H, *H-4*), 3.95 (dd, 1 H, $J_{4,5a}$ = 4.3 Hz, $J_{5a,5b}$ = 12.9 Hz, *H-5a*), 3.75 (dd, 1 H, $J_{4,5b}$ = 3.8 Hz, *H-5b*), 5.14 (s, 1 H, *H-1′*), 5.16 (s, 1 H, *H-2′*), 4.94 (d, 1 H, $J_{3,4}$ = 4.3 Hz, *H-3′*), 4.26–4.30 (m, 1 H, *H-4′*), 4.45 (dd, 1 H, $J_{4,5a}$ = 3.9 Hz, $J_{5a,5b}$ = 12.9 Hz, *H-5′a*), 4.23 (dd, 1 H, $J_{4,5b}$ = 4.7 Hz, *H-5′b*), 3.76 (s, 3 H, -OCH_3), 2.09–2.18 (s, 15 H, C$H_{3acetyl}$), 6.78–7.02 (m, 4 H, -Ph). ^{13}C NMR (500 MHz, $CDCl_3$): 104.49 (C-1), 81.74 (C-2), 76.04 (C-3), 81.38 (C-4), 64.87 (C-5), 105.18 (C-1′), 80.68 (C-2′), 76.96 (C-3′), 80.49 (C-4′), 62.96 (C-5′), 55.31 (-OCH_3), 20.30, 20. 43, 20.48 ($CH_{3acetyl}$), 169.11, 169.50, 169.85, 169.93, 170,26 (CH_3CO-), 114.25, 117.97 (-Ph), 149.72, 154.89 (-Ph_{quat}).
$[\alpha]_D$: –30.35 (c 0.32, $CHCl_3$). MALDI-TOF measurements: 621.51 $[M + Na]^+$

(20) Spectroscopic data of compound **17**: ^1H NMR (500 MHz, $CDCl_3$): δ 5.42 (d, 1 H, $J_{1,2}$ = 5.2 Hz, A-1), 4.24–4.27 (m, 2 H, A-2, B-3), 4.56 (d, 1 H, $J_{3,4}$ = 2.3 Hz, A-3), 4.18 (dd, 1 H, $J_{4,5}$ = 8 Hz, A-4), 3.94–3.98 (m, 1 H, A-5), 3.58 (d, 1 H, A-6a), 4.13 (dd, 1 H, $J_{5,6b}$ = 2.3 Hz, $J_{6a,6b}$ = 10.3 Hz, A-6b), 4.26 (d, 1 H, $J_{1,2}$ = 8.5 Hz, B-1), 3.81 (dd, 1 H, $J_{2,3}$ = 6 Hz, B-2), 4.03 (dd, 1 H, $J_{3,4}$ = 1.8 Hz, B-4), 3.92–3.95 (m, 1 H, B-5), 3.92–3.96 (m, 1 H, B-6a), 4.03–4.06 (m, 1 H, B-6b), 4.69 (d, 1 H, $J_{1,2}$ = 8 Hz, C-1), 5.22 (dd, 1 H, $J_{2,3}$ = 10 Hz, C-2), 4.98 (dd, 1 H, $J_{3,4}$ = 3.5 Hz, C-3), 5.36 (dd, 1 H, $J_{4,5}$ = 1.1 Hz, C-4), 3.89–3.93 (m, 1 H, C-5), 4.13–4.16 (m, 2 H, C-6a, C-6b), 4.17 (s, 1 H, D-1), 5.08 (s, 1 H, D-2), 5.15 (d, 1 H, $J_{3,4}$ = 1.6 Hz, D-3), 4.56–4.5 (m, 1 H, D-4), 4.14 (d, 1 H, $J_{4,5a}$ = 1.1 Hz, D-5a), 3.58 (dd, 1 H, $J_{4,5ab}$ = 1.6 Hz, $J_{5a,5b}$ = 9.8 Hz, d-5b), 5.46 (s, 1 H, E-1), 5.18 (s, 1 H, E-2), 4.91 (dd, 1 H, *J* = 4.9 Hz, *J* = 0.8 Hz, E-3), 4.29–4.34 (m, 1 H, E-4), 4.44 (dd, 1 H, $J_{4,5a}$ = 3.1 Hz, $J_{5a,5b}$ = 11.8 Hz, E-5a), 4.21–4.24 (m, 1 H, E-5b), 1.26, 1.30, 1.31, 1.36, 1.49, 1.53 (s, 24 H, CH_{3ip}), 1.95, 2.04, 2.06, 2.07, 2.08, 2.09, 2.10, 2.12, 2.15 (C$H_{3acetyl}$). ^{13}C NMR (200 MHz, $CDCl_3$): 96.00 (A-1), 69.91 (A-2), 70.74 (A-3), 71.40 (A-4), 67.04 (A-5), 69.55 (A-6), 101.07 (B-1), 74.08 (B-2), 79.84 (B-3), 73.75 (B-4), 70.74 (B-5), 68.62 (B-6), 101.30 (C-1), 68.75 (C-2), 70.74 (C-3), 67.04 (C-4), 70.74 (C-5), 61.13 (C-6), 104.91 (D-1), 80.84 (D-2), 77.63 (D-3), 80.98 (D-4), 63.35 (D-5), 104.91 (E-1), 81.34 (E-2), 76.13 (E-3), 81.84 (E-4), 64.87 (E-5), 20.58, 20.75 ($CH_{3acetyl}$), 24.08, 24.73, 25.87, 26.00, 26.29, 27.94, 29.19, 30.83 (CH_{3ip}), 108.12, 108.85, 110.57 (C_{quat}), 169.27, 169.35, 169.80, 169.88, 170.15, 170.27, 170.36, 170.48 (CH_3CO).
$[\alpha]_D$: -66.18 (c 0.3173, $CHCl_3$). MALDI-TOF measurements: 1289.87 $[M + Na]^+$

(21) Spectroscopic data of compound **1**: ^1H NMR (500 MHz, D_2O): δ 5.21 (A-1α, $J_{1,2}$ = 3.8 Hz), 4.53 (A-1β, $J_{1,2}$ = 7.9 Hz), 3.41 (A-2β), 3.60 (A-3β), 4.49 (B-1, $J_{1,2}$ = 7.8 Hz), 3.58 (B-2), 3.72 (B-3), 4.40 (C-1, $J_{1,2}$ = 7.8 Hz), 3.44 (C-2), 3.60 (C-3), 5.24 (D-1, $J_{1,2}$ = 3.7 Hz), 4.12 (D-2), 3.90 (D-3), 4.17 (D-4), 3.67 and 3.75 (D-5a and d-5b).
^{13}C NMR (500 MHz, H_2O): 92.07 (A-1α), 96.09 (A-1ι), 71.52 (A-2ι), 72.39 (A-3β), 69.16 (A-6β), 101.61 (B-1), 75.66 (B-2), 72.49 (B-3), 68.73 (B-6), 103.07 (C-1), 70.43 (C-2), 72.41 (C-3), 60.69 (C-6), 107.93 (D-1), 80.64 (D-2), 76.57 (D-3), 83.95 (D-4), 61.07 (D-5).
$[\alpha]_D$: –1.55 (c 0.1931, H_2O).MALDI-TOF measurements: 657.14 $[M + Na]^+$

(22) Spectroscopic data of compound **2**: ^1H NMR (500 MHz, D_2O): δ 5.20 (A-1α, $J_{1,2}$ = 3.98 Hz,), 4.52 (A-1β, $J_{1,2}$ = 8.16 Hz), 3.72 (A-2α), 3.44 (A-2ι), 3.59 (A-3), 3.91 (A-4), 4.48 (B-1, $J_{1,2}$ = 8.16 Hz), 3.59 (B-2), 3.71 (B-3), 3.91 (B-4), 4.40 (C-1, $J_{1,2}$ = 8.16 Hz), 3.48 (C-2), 3.59 (C-3), 3.87 (C-4), 5.03 (D-1, $J_{1,2}$ = 2.39 Hz,), 4.08 (D-2), 5.23 (E-1), 4.12 (E-2).
^{13}C NMR (500 MHz, H_2O): 92.08 (A-1α), 96.10 (A-1β), 71.55 (A-2), 72.41 (A-3), 60.69 (A-6), 101.69 (B-1), 75.60 (B-2), 74.85 (B-3), 102.84 (C-1), 70.43 (C-2), 72.35 (C-3), 69.17 and 68.73 (B-6 and C-6), 107.10 (D-1), 80.60 (D-2), 76.24 (D-3), 82.25 (D-4), 66.55 (D-5), 107.98 (E-1), 80.60 (E-2), 76.90 (E-3), 83.63 (E-4), 60.89 (E-5).
MALDI-TOF measurements:791.13 $[M + Na]^+$.

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