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Note

Cross metathesis for the synthesis of novel C-sialosides

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Abstract—Cross metathesis of both anomers of *C*-allyl sialoside (**3α/3β**) with styrene catalyzed by the second generation Grubbs or Hoveyda-Grubbs catalysts gave the corresponding aryl derivatives (**4α/4β**) in virtually quantitative yields. The products were hydrogenated to model compounds **5α/5β**. Similarly, reaction of the α-anomer **3α** with galactose derivative **8** gave the olefin-linked disaccharide mimetic **9**. Following hydrogenation and deprotection, the ethylene-bridged Neu5Acα(2→6)Gal analogue **11** could be obtained.

Keywords: Cross metathesis; Ruthenium catalyst; C-Sialosides; Disaccharide mimetics

With the discovery of the important roles that oligosaccharide structures play in cell biology, the enzymes dealing with those structures also became of increasing interest to research. Revealing the properties of glycosyltransferases as well as glycosidases not only enhances the understanding of the mechanisms underlying infections but also leads to effective new strategies in defeating them. Of particular interest are the enzymes belonging to the sialidase family (EC 3.2.1.18), because *N*-acetyl neuraminic acid (Neu5Ac) plays a pivotal role in nature as a major constituent of a variety of glycoconjugates such as oligosaccharides, glycoproteins and gangliosides that occur in animals and several pathogens.[1] In most cases, neuraminic acids are terminally α-(2→3) or α-(2→6)-linked to a galactose of the oligosaccharide cell epitope. Due to the terminal position of Neu5Ac in these oligosaccharide scaffolds, sialylation is associated with many processes, such as cell recognition and cell differentiation. In some biological events, the Neu5Ac allows recognition by a suitable receptor protein; in others, the presence of Neu5Ac is able to mask recognition sites.

In the organism causing South American trypanosomiasis[2] (Chagas disease), *Trypanosoma cruzi*, a *trans*-sialidase (TcTS) causes the transfer of Neu5Ac from a human host cell to the cell epitope of the pathogen.[3] This unusual transfer mechanism enables the pathogen to protect its own cell surface against recognition by the human immune system. Because *T. cruzi* trypomastigotes lacking *trans*-sialidase are less efficient in cell invasion,[4] this enzyme seems to play a crucial role in *T. cruzi* infection. For the investigation of TcTS, we are interested in substrate analogues that are stable to hydrolysis by the enzyme to use in affinity measurements. We envisage C-glycosides of Neu5Ac to meet those demands.

Previous syntheses of C-sialosides employed strategies such as alkylation of 2-deoxy derivatives,[5] samarium-mediated coupling of carbonyl compounds to 2-sulfonyl derivatives[6] and even de novo synthesis of the C-glycosidically linked Neu5Ac scaffold.[7] Previous studies by Paulsen and Matschulat reported on a radical-induced allylation at the anomeric centre of Neu5Ac, which opens up possibilities for further derivatization.[8] We decided to focus on cross metathesis reactions, employing both the Grubbs catalyst (second generation) **1** and the Hoveyda-Grubbs catalyst (second generation) **2** (Fig. 1).

Olefin metathesis is already established as a method in carbohydrate synthesis. Applications cover fields such as homodimerization and linking of allyl glycosides, cross

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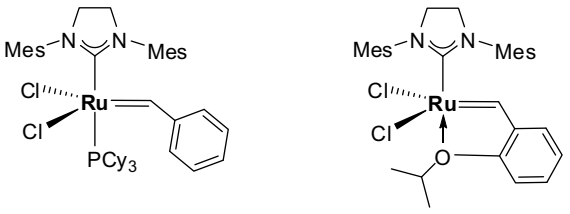


1 2

Figure 1.

metathesis of allyl glycosides and allyl C-glycosides as well as de novo synthesis of carbohydrates and related structures.[9–13] By dimerization of a methylvinyl C-arabinoside, an analogue of a mycobacterial cell wall disaccharide motif could be synthesized.[14] Sialosides were successfully converted to divalent derivatives by olefin metathesis.[15] The synthesis of disaccharide mimetics by olefin metathesis[16] is of particular interest for the reasons mentioned above. Therefore, we investigated the potential of cross metathesis in the syntheses of substrate analogues resistant to hydrolysis by sialic acid related enzymes.

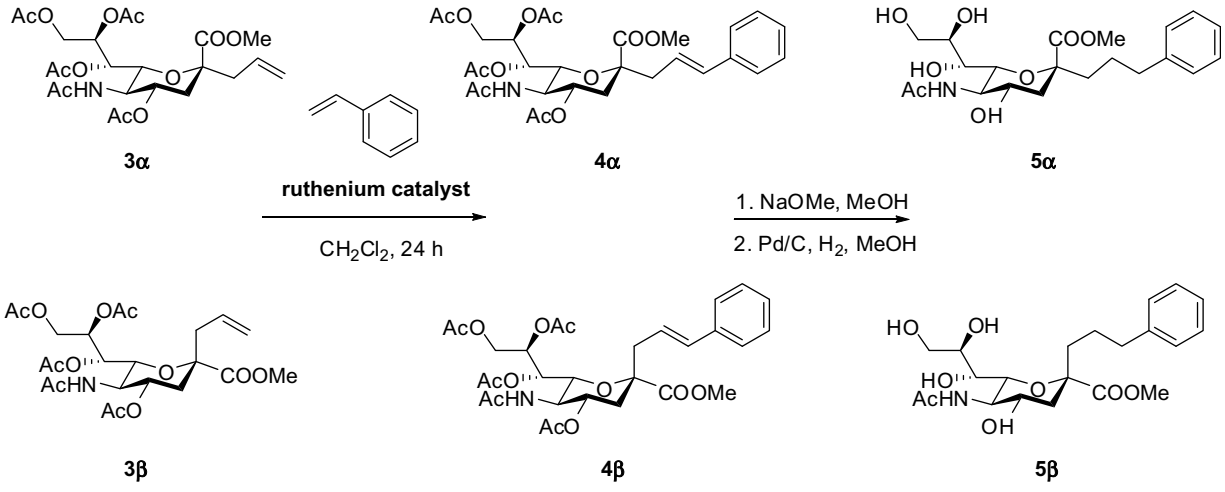
The difficulty in designing a sufficient protocol for cross metathesis reaction with allyl C-sialosides **3α** and **3β**[8] is the homodimerization that results from self metathesis of the substrate. To avoid self metathesis, the reaction was conducted in dilute solutions of the C-sialosides (0.01 M) with the use of an 8–10-fold excess of reactant. Under these conditions, in none of the cases was the formation of any dimerization product of the C-sialoside observed. For the isolation of the cross metathesis product, it was helpful to choose as a reactant a much more nonpolar compound that can easily be separated from the desired products.

First, the reaction conditions were optimized by reacting the allyl C-sialosides **3α** and **3β** with styrene, which meets the above mentioned demands as a reactant, but might also have the ability to mimic sugar moieties.[17] Cross metatheses were conducted with catalysts **1** and **2** in dichloromethane at room temperature and under refluxing conditions (Table 1). Isolated yields show the importance of refluxing conditions for shifting the equilibrium towards the desired products. By using styrene as a reactant in each case, the formation of (*E*)-isomers was observed in excellent yields. It may be assumed that the products are isomerized by subsequent cross metathesis steps to yield the thermodynamically more stable *trans*-olefins. Compounds **4α** and **4β** were deacetylated and hydrogenated to give the saturated C-sialosides **5α** and **5β** (Scheme 1).

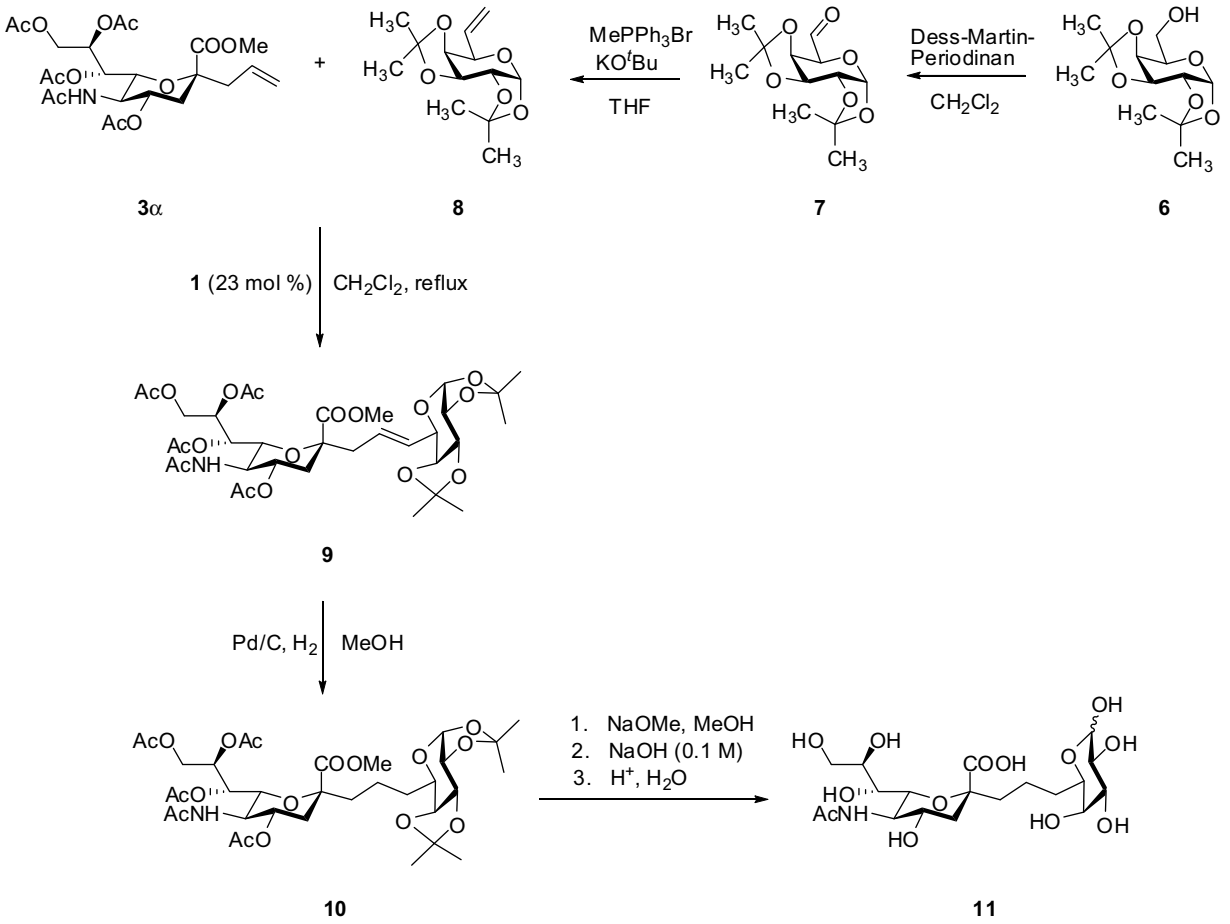
Having established the optimal conditions for cross metathesis, the use of a carbohydrate based olefin as reactant in cross metathesis with allyl C-sialosides was explored. The readily available galactose derivative **6** could be oxidized easily by the method of Dess and Martin[18] to give **7** in 63% yield. Wittig olefination

Table 1.

Ruthenium catalyst (mol %)	Temperature (°C)	Product	Yield (%)
3α + styrene —(ruthenium catalyst, CH_2Cl_2, 24 h)→ **4α**			
1 (8)	25	**4α**	34
1 (5)	40	**4α**	92
2 (16)	25	**4α**	17
2 (24)	40	**4α**	94
3β + styrene —(ruthenium catalyst, CH_2Cl_2, 24 h)→ **4β**			
1 (12)	40	**4β**	100



Scheme 1.



Scheme 2.

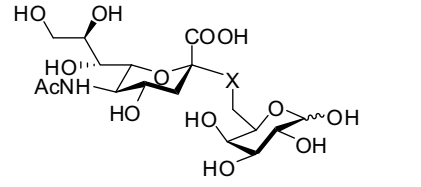


Figure 2.

resulted in the formation of the terminal olefin **8**,[19] which could undergo cross metathesis with **3α** to give the novel derivative **9** in 95% yield (Scheme 2). In this case, the lower reactivity of the internal double bond in C-sialoside **9** leads to a (*E*/*Z*)-ratio of 3:1 (based on NMR). The bulky and nonpolar isopropylidene protecting groups in the reactant facilitated the isolation of the product in a similar manner as described above. Hydrogenation under standard conditions and deprotection (Scheme 2) resulted in an ethylene bridged C-analogue of α-(2→6)-linked sialyl galactose derivative (Fig. 2).

This reaction sequence is short and displayed high yields as well as easy purification steps. Therefore, it should be advantageously applied in the synthesis of a series of various substrate analogues as potential inhibitors or modulators of sialic acid processing enzymes.

1. Experimental

1.1. General methods

Commercially available starting materials were used without further purification. Solvents were dried according to standard methods. TLC was performed on precoated aluminum plates (Silica Gel 60 F_{254}, Merck 5554) employing UV-absorption and charring with 10% H_2SO_4 in ethanol for visualization. For column chromatography Silica Gel 60, 230–400 mesh, 40–63 μm (Merck) was used. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AMX-400 (400 MHz for ^{1}H, 100.6 MHz for ^{13}C) and on Bruker DRX-500 (500 MHz for ^{1}H, 125.8 MHz for ^{13}C) at 300 K. Chemical shifts were calibrated to solvent residual peaks.[20]

The signals were assigned by ^{1}H–^{1}H-COSY, HSQC, HMBC and if necessary NOESY experiments. Optical rotations were measured using a Perkin Elmer 241 (546 nm) or a Krüss Optronic P8000 (589 nm) at 20 °C. MALDI-TOF-MS was performed on a Bruker Biflex III with dihydroxybenzoic acid or trihydroxyanthracene as matrices in positive reflector mode. ESI-HRMS was performed on a Thermo Finnigan MAT 95 XL mass spectrometer.

Compound **7** was synthesized using the protocol of Dess and Martin.[18] Compound **8** was synthesized following a standard procedure. The NMR data of **7** and **8** were consistent with the published data.[21]

1.2. General procedure for cross metathesis with C-sialosides (GP1)

The allyl C-sialoside (0.35 mmol) was dissolved under nitrogen in anhydrous dichloromethane (35 mL) to yield an 0.01 M solution and the second reactant (8–10-fold excess) was added. After the addition of the ruthenium catalyst, the reaction was stirred at the indicated temperature (Table 1) for 24 h. The removal of the solvent in vacuo and column chromatography on silica gel (toluene–acetone) yielded the product as a white foam.

1.3. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-2-*C*-(3-phenylprop-2-enyl)-D-*erythro*-L-*manno*-nononate (4α)

Various amounts of **3α** were reacted with the indicated catalyst amounts at the given temperature (Table 1) following GP1 to give **4α** (yield, see Table 1): $[\alpha]_{546}^{20}$ −8.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.39–7.19 (m, 5H, arom. H), 6.38 (d, 1H, $J_{CH,CH}$ = 16.1 Hz, Ph–CH=), 6.19–6.11 (m, 1H, =CH–), 5.41 (ddd, 1H, $J_{8,7}$ = 6.9 Hz, $J_{8,9b}$ = 6.0 Hz, $J_{8,9a}$ = 2.5 Hz, H-8), 5.34 (dd, 1H, $J_{7,8}$ = 6.9 Hz, $J_{7,6}$ = 1.6 Hz, H-7), 5.19 (br d, 1H, NH), 4.88–4.80 (m, 1H, H-4), 4.43 (dd, 1H, $J_{9a,9b}$ = 12.3 Hz, $J_{9a,8}$ = 2.5 Hz, H-9a), 4.15 (dd, 1H, $J_{9b,9a}$ = 12.3 Hz, $J_{9b,8}$ 6.0 Hz, H-9b), 4.08–3.98 (m, 2H, H-5, H-6), 3.72 (s, 3H, OCH_3), 2.69–2.57 (m, 2H, CH_2), 2.52 (dd, 1H, $J_{3,3}$ = 12.8 Hz, $J_{3,4}$ = 4.5 Hz, H-3_{eq}), 2.12 (s, 3H, $COCH_3$), 2.11 (s, 3H, $COCH_3$), 2.01 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 1.87 (s, 3H, $COCH_3$), 1.84 (dd, 1H, $J_{3,3}$ = 12.8 Hz, $J_{3,4}$ = 12.3 Hz, H-3_{ax}); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 171.72, 171.20, 170.85, 170.44, 170.34, 170.22 (C=O), 137.06 (arom. C_{quart}), 134.27 (Ph–CH=), 128.61, 127.60, 126.51 (arom. C), 122.76 (=CH–), 80.84 (C-2), 73.69 (C-6), 70.31 (C-4), 69.93 (C-8), 68.09 (C-7), 62.56 (C-9), 52.55 (OCH_3), 49.75 (C-5), 43.66 (CH_2), 37.52 (C-3), 23.32, 21.24, 21.03, 20.92, 20.90 (5 × CH_3). MALDI-TOF: *m/z* 592.4 $[M+H]^+$, 614.3 $[M+Na]^+$, 630.3 $[M+K]^+$. ESIMS: 614.2189 $[M+Na]^+$, calcd: 614.2213.

1.4. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-2-*C*-(3-phenylprop-2-enyl)-D-*erythro*-L-*gluco*-nononate (4β)

Compound **3β** (250 mg; 485 μmol) was reacted with styrene (0.5 mL, 4 mmol) and catalyst **1** (50 mg 59 μmol, 12 mol %) following GP1 to give **4β** (287 mg; 100%): $[\alpha]_D^{20}$ −12 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.33–7.15 (m, 5H, arom. H), 6.51 (d, 1H, $J_{CH,CH}$ = 15.8 Hz, Ph–CH=), 5.99–5.92 (m, 1H, =CH–), 5.38–5.37 (dd, 1H, $J_{7,8}$ = 2.5 Hz, $J_{7,6}$ = 2.2 Hz, H-7), 5.34–5.28 (m, 2H, H-4, NH), 5.11 (ddd, 1H, $J_{8,9b}$ = 7.9 Hz, $J_{8,7}$ = 2.5 Hz, $J_{8,9a}$ = 2.2 Hz, H-8), 4.76 (dd, 1H, $J_{9a,9b}$ = 12.3 Hz, $J_{9a,8}$ = 2.2 Hz, H-9a), 4.13 (dd, 1H, $J_{9b,9a}$ = 12.3 Hz, $J_{9b,8}$ = 7.9 Hz, H-9b), 4.10 (dd, 1H, $J_{6,5}$ = 10.7 Hz, $J_{6,7}$ = 2.2 Hz, H-6), 3.87 (m, 1H, H-5), 3.76 (s, 3H, OCH_3), 3.00–2.94 (m, 1H, CH_2), 2.77–2.72 (m, 1H, CH_2), 2.38 (dd, 1H, $J_{3,3}$ = 13.2 Hz, $J_{3,4}$ = 5.1 Hz, H-3_{eq}), 2.14 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.02 (s, 3H, $COCH_3$), 1.96 (dd, 1H, $J_{3,3}$ = 13.2 Hz, $J_{3,4}$ = 11.9 Hz, H-3_{ax}), 1.90 (s, 3H, $COCH_3$), 1.85 (s, 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 171.65, 171.10, 170.95, 170.69, 170.64, 170.41 (C=O), 136.79 (arom. C_{quart}), 134.68 (Ph–CH=), 128.62, 127.75, 126.49 (arom. C), 122.14 (=CH–), 79.65 (C-2), 73.13 (C-6), 72.00 (C-8), 69.21 (C-7), 69.26 (C-4), 62.75 (C-9), 52.71 (OCH_3), 50.51 (C-5), 36.16, 36.13 (C-3, CH_2), 23.45, 21.12, 21.07, 20.96, 20.75 (5 × CH_3). MALDI-TOF: *m/z* 592.1 $[M+H]^+$, 614.1 $[M+Na]^+$, 630.1 $[M+K]^+$. ESIMS: 614.2207 $[M+Na]^+$, calcd: 614.2213.

1.5. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-2-*C*-(3-phenylpropyl)-D-*erythro*-L-*manno*-nononate (5α)

Compound **4α** (83 mg; 0.14 mmol) was dissolved in methanolic sodium methoxide solution (20 mL, 0.1 M) and stirred for 5 h at room temperature. The solution was then neutralized with Dowex 50X8 (H^+) resin, filtrated and concentrated. The residue was hydrogenated with a catalytic amount of palladium on charcoal in MeOH (20 mL) under a hydrogen atmosphere. Filtration, evaporation of the solvent and flash chromatography (CH_2Cl_2–MeOH) gave **5α** (60 mg, 100%): $[\alpha]_D^{20}$ −172.4 (*c* 1, MeOH); ^{1}H NMR (CD_3OD, 400 MHz): δ 7.27–7.12 (m, 5H, arom. H), 3.85–3.80 (m, 2H, H-8, H-9a), 3.75 (s, 3H, OCH_3), 3.72–3.54 (m, 3H, H-4, H-5, H-9b), 3.49 (dd, 1H, $J_{6,5}$ = 7.5 Hz, $J_{6,7}$ = 1.5 Hz, H-6), 3.47 (dd, 1H, $J_{7,8}$ = 6.0 Hz, $J_{7,6}$ = 1.5 Hz, H-7), 2.65–2.53 (m, 3H, CH_2, H-3_{eq}), 1.99 (s, 3H, $COCH_3$), 1.81–1.68 (m, 3H, CH_2), 1.60–1.47 (m, 2H, CH_2, H-3_{ax}); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 175.62, (C=O), 143.10 (arom. C_{quart}), 129.42, 129.38, 126.90 (arom. C), 81.59 (C-2), 75.78 (C-6), 72.88 (C-8), 70.20 (C-7), 69.09 (C-4), 64.61 (C-9), 54.19 (C-5), 53.06

(OCH_3), 42.11 (CH_2), 40.86 (C-3), 36.58 (CH_2), 26.44 (CH_2), 22.61 (CH_3). MALDI-TOF: *m/z* 426.2 $[M+H]^+$, 448.2 $[M+Na]^+$, 464.2 $[M+K]^+$. ESIMS: 448.1943 $[M+Na]^+$, calcd: 448.1947.

1.6. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-2-*C*-(3-phenylpropyl)-D-*erythro*-L-*gluco*-nononate (5β)

Compound **4β** (70 mg; 0.12 mmol) was dissolved in methanolic sodium methoxide solution (20 mL, 0.1 M) and stirred for 5 h at room temperature. The solution was then neutralized with Dowex 50X8 (H^+) resin, filtrated and concentrated. The residue was hydrogenated with a catalytic amount of palladium on charcoal in MeOH (20 mL) under a hydrogen atmosphere. Filtration, evaporation of the solvent and flash chromatography (CH_2Cl_2–MeOH) gave **5β** (46 mg, 92%): $[\alpha]_D^{20}$ −33.6 (*c* 1, MeOH); ^{1}H NMR (CD_3OD, 400 MHz): δ 7.27–7.12 (m, 5H, arom. H), 3.93 (ddd, 1H, $J_{4,5} = 11.3$ Hz, $J_{4,5} = 9.3$ Hz, $J_{4,3} = 4.8$ Hz, H-4), 3.85–3.71 (m, 4H, C-5, C-6, C-8, C-9a), 3.70 (s, 3H, OCH_3), 3.69–3.43 (m, 2H, C-7, C-9b), 2.69–2.51 (m, 2H, CH_2), 2.19 (dd, 1H, $J_{3,3} = 13.1$ Hz, $J_{3,4} = 4.8$ Hz, H-3_{eq}), 2.16–2.09 (m, 1H, CH_2), 1.99 (s, 3H, $COCH_3$), 1.95–1.85 (m, 1H, CH_2), 1.78–1.70 (m, 1H, CH_2), 1.62 (dd, 1H, $J_{3,3} = 13.1$ Hz, $J_{3,4} = 11.3$ Hz, H-3_{ax}), 1.35–1.24 (m, 1H, CH_2); ^{13}C NMR ($CDCl_3$, 100.6 MHz, carbonyl groups not detected): δ 129.48, 129.35, 126.85 (arom. C), 80.82 (C-2), 71.94, 71.75 (C-6, C-8), 70.60 (C-7), 68.12 (C-4), 65.24 (C-9), 54.48 (C-5), 52.96 (OCH_3), 41.38 (C-3), 36.56, 32.42, 25.90 (3 × CH_2), 22.84 (CH_3). MALDI-TOF: *m/z* 426.2 $[M+H]^+$, 448.2 $[M+Na]^+$, 464.2 $[M+K]^+$. ESIMS: 448.1948 $[M+Na]^+$, calcd: 448.1947.

1.7. Methyl 5′-acetamido-4′,7′,8′,9′-tetra-*O*-acetyl-2′,6′-anhydro-3′,5′-dideoxy-2′-*C*-(6,7,8-trideoxy-1,2;3,4-di-*O*-isopropylidene-α-D-*galacto*-oct-6-enopyranos-8-yl)-D-*erythro*-L-*manno*-nononate (9)

Compound **3α** (75 mg; 0.15 mmol) was reacted with **8** (240 mg; 0.936 mmol) and catalyst **1** (29 mg, 34 μmol; 22 mol %) according to GP1 (refluxing conditions) to give **9** (106 mg, 95%) as a colourless oil: $[\alpha]_{546}^{20}$ −45 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz, major isomer is characterized): δ 5.73–5.59 (m, 2H, H-6^I, H-7^I), 5.52 (d, 1H, $J_{1,2} = 5.1$ Hz, H-1^I), 5.37–5.30 (m, 2H, H-8^{II}, H-7^{II}), 5.18 (br d, 1H, NH), 4.85–4.77 (m, 1H, H-4^{II}), 4.59 (dd, 1H, $J_{3,2} = 7.9$ Hz, $J_{3,4} = 2.3$ Hz, H-3^I), 4.34 (dd, 1H, $J_{9a,9b} = 12.4$ Hz, $J_{9a,8} = 2.6$ Hz, H-$9a^{II}$), 4.31–4.24 (m, 2H, H-2^I, H-5^I), 4.20 (dd, 1H, $J_{4,3} = 7.9$ Hz, $J_{4,5} = 1.9$ Hz, H-4^I), 4.08 (dd, 1H, $J_{9b,9a} = 12.4$ Hz, $J_{9b,8} = 5.6$ Hz, H-$9b^{II}$), 4.01–3.97 (m, 2H, H-5^{II}, H-6^{II}), 3.71 (s, 3H, OCH_3), 2.52–2.43 (m, 2H, H-3^{II}_{eq}, H-8^I), 2.11 (s, 6H, 2 × $COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 1.86 (s, 3H, $COCH_3$), 1.77 (dd, 1H, $J_{3,3} = 12.5$ Hz, $J_{3,4} = 12.2$ Hz, H-3^{II}_{ax}), 1.57 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 1.32 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 171.58, 171.18, 170.80, 170.32 170.07 (C=O), 130.91, 126.91 (C-6^I, C-7^I), 109.14, 108.55 ($C(CH_3)_2$), 96.51 (C-1^I), 80.45 (C-2^{II}), 73.76 (C-6^{II}), 73.31 (C-4^I), 70.94, 70.43 (C-2^I, C-3^I), 70.37 (C-4^{II}), 69.12 (C-8^{II}), 68.70 (C-7^{II}), 67.80 (C-5^I), 62.45 (C-9^{II}), 52.54 (OCH_3), 49.73 (C-5^{II}), 42.95 (C-8^I), 37.49 (C-3^{II}), 26.18, 26.10, 25.05, 24.45 (CCH_3), 23.32, 21.19, 21.02 (3 × CH_3), 20.93 (2 × CH_3). MALDI-TOF: *m/z* 766.3 $[M+Na]^+$, 782.3 $[M+K]^+$.

1.8. Methyl 5′-acetamido-4′,7′,8′,9′-tetra-*O*-acetyl-2′,6′-anhydro-3′,5′-dideoxy-2′-*C*-(6,7,8-trideoxy-1,2;3,4-di-*O*-isopropylidene-α-D-*galacto*-octopyranos-8-yl)-D-*erythro*-L-*manno*-nononate (10)

Compound **9** (65 mg; 87 μmol) was hydrogenated with a catalytic amount of palladium on charcoal in MeOH (20 mL) under a hydrogen atmosphere. Filtration, evaporation of the solvent and flash chromatography (toluene–acetone) gave **10** (62 mg, 95%): $[\alpha]_D^{20}$ −50 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.50 (d, 1H, $J_{1,2} = 5.1$ Hz, H-1^I), 5.36–5.29 (m, 2H, H-8^{II}, H-7^{II}), 5.13 (br d, 1H, NH), 4.84–4.77 (m, 1H, H-4^{II}), 4.57 (dd, 1H, $J_{3,2} = 7.9$ Hz, $J_{3,4} = 2.0$ Hz, H-3^I), 4.35 (dd, 1H, $J_{9a,9b} = 12.2$ Hz, $J_{9a,8} = 2.5$ Hz, H-$9a^{II}$), 4.27 (dd, 1H, $J_{2,1} = 5.1$ Hz, $J_{2,3} = 2.0$ Hz, H-2^I), 4.14 (dd, 1H, $J_{4,3} = 7.9$ Hz, $J_{4,5} = 1.8$ Hz, H-4^I), 4.11 (dd, 1H, $J_{9b,9a} = 12.2$ Hz, $J_{9b,8} = 5.4$ Hz, H-$9b^{II}$), 4.02–3.94 (m, 2H, H-5^{II}, H-6^{II}), 3.74 (s, 3H, OCH_3), 3.70–3.65 (m, 1H, H-5^I), 2.49 (dd, 1H, $J_{3,3} = 12.7$ Hz, $J_{3,4} = 4.6$ Hz, H-3^{II}_{eq}), 2.12 (s, 6H, 2 × $COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.02 (s, 3H, $COCH_3$), 1.87 (s, 3H, $COCH_3$), 1.79–1.62 (m, 7H, H-3^{II}_{ax}, H-6^I, H-7^I, H-8^I), 1.53 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.32 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100.6 MHz, carbonyl groups were not detected): δ 109.04, 108.39 ($C(CH_3)_2$), 96.63 (C-1^I), 80.49 (C-2^{II}), 73.34 (C-6^{II}), 72.76 (C-4^I), 71.03 (C-3^I), 70.75 (C-2^I), 70.44 (C-4^{II}), 69.41 (C-8^{II}), 67.92 (C-7^{II}), 67.29 (C-5^I), 62.40 (C-9^{II}), 52.49 (OCH_3), 49.94 (C-5^{II}), 39.83, 38.00, 30.20 (C-3^{II}, C-6^I, C-8^I), 26.20, 26.16, 25.12, 24.42 (CCH_3), 23.39, 22.09, 21.07, 20.98, 20.96 (5 × CH_3), 19.56 (C-7^I). MALDI-TOF: *m/z* 746.3 $[M+H]^+$, 768.3 $[M+Na]^+$, 784.3 $[M+K]^+$. ESIMS: 768.3065 $[M+Na]^+$, calcd: 768.3055.

1.9. 5′-Acetamido-2′,6′-anhydro-3′,5′-dideoxy-2′-*C*-(6,7,8-trideoxy-D-*galacto*-octopyranos-8-yl)-D-erythro-L-*manno*-nonulosonic acid (11)

Compound **10** (62 mg, 83 μmol) was dissolved in methanolic sodium methoxide solution (20 mL, 0.1 M) and stirred for 5 h at room temperature. The solution was then neutralized with Dowex 50X8 (H^+) resin, filtrated

and concentrated. The residue was dissolved in water and stirred with Dowex 50X8 (H^+) resin overnight at 60 °C. After filtration, the solution was treated with 0.1 M sodium hydroxide solution. After stirring for 2 h, the solution was again neutralized with Dowex 50X8 (H^+) resin, filtrated, purified over Biogel P2 and lyophilized to give **11** (24 mg, 60%): mp 162–164 °C (decomp.); $[\alpha]_D^{20}$ +12.4 (*c* 1, D_2O); ^1H NMR (D_2O, 500 MHz): δ 5.22 (d, 0.3H, $J_{1,2} = 3.8$ Hz, H-$1^I\alpha$), 4.55 (d, 0.7H, $J_{1,2} = 7.8$ Hz, H-$1^I\beta$), 4.06–3.56 (m, 10.3H, H-$2^I\alpha$, H-3^I, H-4^I, H-5^I, H-4^{II}, H-5^{II}, H-6^{II}, H-7^{II}, H-8^{II}, H-9^{II}) 3.47 (dd, 0.7H, $J_{2,3} = 9.8$ Hz, $J_{2,1} = 7.8$ Hz, H-$2^I\beta$), 2.63 (dd, 1H, $J_{3,3} = 12.8$ Hz, $J_{3,4} = 4.5$ Hz, H-3^{II}_{eq}), 2.06 (s, 3H, $COCH_3$), 1.80–1.32 (m, 7H, H-3^{II}_{ax}, H-6^I, H-7^I, H-8^I); ^{13}C NMR (D_2O, 100.6 MHz): δ 175.45 (C=O), 96.68 (C-$1^I\beta$), 92.54 (C-$1^I\alpha$), 82.21 (C-2^{II}), 74.78, 73.81, 73.44, 72.42, 72.27, 71.05, 70.46, 69.80, 69.33, 68.69 (C-2^I, C-3^I, C-4^I, C-5^I, C-4^{II}, C-6^{II}, C-7^{II}, C-8^{II}), 63.07 (C-9^{II}), 52.78 (C-5^{II}), 41.34 (C-3^{II}), 39.87, 30.08 (C-6^I, C-8^I), 22.36 (CH_3), 19.97 (C-7^I). MALDI-TOF: *m/z* 484.4 $[M+H]^+$, 506.4 $[M+Na]^+$, 522.3 $[M+K]^+$. ESIMS: 506.1849 $[M+Na]^+$, calcd: 506.1850.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2008.03.036.

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