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PAPER

Sialic acid *C*-glycosides with aromatic residues: Investigating enzyme binding and inhibition of *Trypanosoma cruzi trans*-sialidase†

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Several α-configured *C*-sialosides were synthesised by cross metathesis and further synthetic derivatisation to obtain ligands for *Trypanosoma cruzi trans*-sialidase (TcTS), a key enzyme in Chagas disease. Affinities of these compounds to immobilised TcTS were measured by surface plasmon resonance (SPR). The K_D values thus obtained are in the lower millimolar range and will be discussed. The results show the importance of addressing Tyr_{119} and Trp_{312} side chains of TcTS in target oriented ligand synthesis, since these amino acids constitute the acceptor binding region in the active site of TcTS. The best ligand showed a significant decrease of TcTS activity in a preliminary NMR based inhibition assay.

Introduction

The important roles that oligosaccharide structures play in cell biology lead to increasing interest in research concerning enzymes involved in synthesis or degradation of such structures. Elucidating the enzyme mechanisms as well as the biological context in which glycosyltransferases and glycosidases display their activity may induce more effective as well as novel approaches to defeat infections. Of particular interest are enzymes belonging to the sialidase family (EC 3.2.1.18), because neuraminic acid (Neu5Ac) plays an important role in Nature as a major constituent of a variety of glycoconjugates occurring in animals and many pathogens.[1] Terminal sialylation of glycans is associated with many processes such as cell recognition and cell differentiation. Either it allows recognition by a suitable receptor protein, or the presence of Neu5Ac is able to mask recognition sites.

In the South American trypanosomiasis (Chagas disease)[2] *trans*-sialidase of *Trypanosoma cruzi* (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. Therefore, development of ligands as potential inhibitors for TcTS can be considered as a most promising goal in defeating *T. cruzi* infection.[5] Although several efforts have been made previously in this area,[6–8] no strong inhibitor for TcTS is known to date.

For investigation of possible ligands for TcTS, analogues of α-sialosides are of particular interest. In this paper a rational synthetic approach is presented, based on qualitative considerations deduced from the crystal structure of TcTS in complex with sialyl lactose published by Amaya *et al.*[9]

Ligand design for TcTS should particularly address the fact that TcTS shows additional binding of acceptor molecules effected by two aromatic amino acid side chains (Tyr_{119} and Trp_{312}, see Fig. 1),[9] responsible for the transfer of Neu5Ac and the negligible amount of hydrolysis occurring in TcTS catalyzed enzymatic reactions.[3]

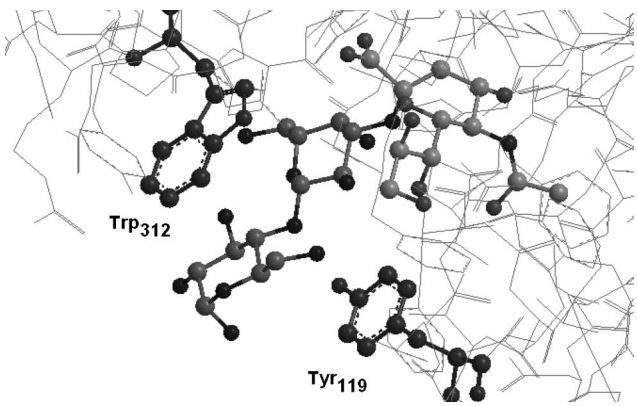

Fig. 1 Tyr_{119} and Trp_{312} in the acceptor binding site (TcTS in complex with sialyl lactose[9]).

Another intended modification consists of adding an aromatic residue to O-9 of the glycerol side chain. The vicinity of Trp_{120} (Fig. 2) suggests a positive effect of this derivatisation.

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† Electronic supplementary information (ESI) available: General methods, synthesis of new compounds, ^1H and ^{13}C NMR spectra. See DOI: 10.1039/c0ob01176b

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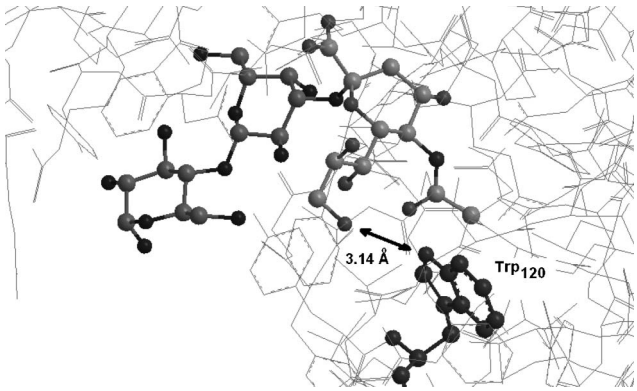

Fig. 2 Trp_{120} contact with the glycerol chain.[9]

Finally, the presence of several arginine residues in the active site would allow for an ionic interaction, especially for Arg_{53} close to O-4 of sialic acid (Fig. 3). Therefore we decided to introduce a carboxylate in this position.

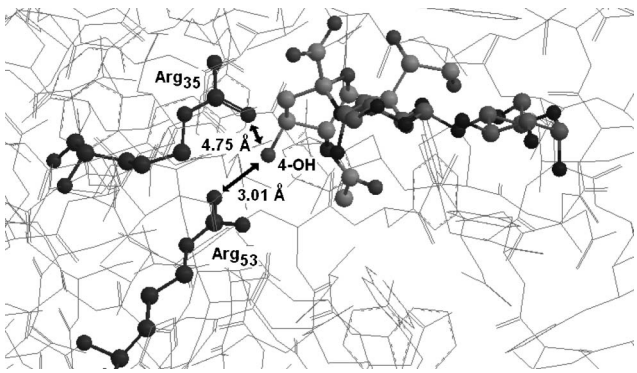

Fig. 3 Arginine side chains in proximity to O-4.

The synthesised compounds should then be studied in SPR affinity measurements with immobilised recombinant *trans*-sialidase. Resulting dissociation constants (K_D) should provide a more detailed picture of structural requirements for TcTS binding, hence enabling the synthesis of competitive inhibitors against Chagas disease.

Results and Discussion

Synthesis of the ligands

Aromatic residues for the acceptor binding site. Allyl *C*-sialosides[10] are useful precursors for the planned derivatisations (Fig. 4). Recently we reported on an approach for adding substituents to allyl-*C*-sialosides by olefin cross metathesis, leading to *C*-sialosides with both aromatic and carbohydrate residues.[11] In this work we employed also *p*-methoxy- and *p*-nitro-substituted styrenes, synthesised by Wittig reaction of the corresponding aldehydes,[12] to further explore this method and fine-tune the electronic properties of the intended ligands. Both the second generation Grubbs catalyst (**1**) and the second generation Grubbs–Hoveyda (**2**) catalyst, were employed in our studies.

Only *trans*-configured cross metathesis product could be obtained with unsubstituted styrene **4a**.[11] This can be explained by

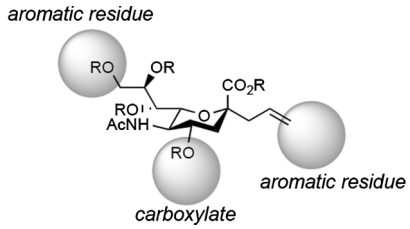

Fig. 4 Intended derivatisations.

equilibration of the intermediate *cis*-product that is supposed to react faster than the thermodynamically more stable *trans*-product.[13]

With the nitro-compound complete conversion was observed, however a 1 : 5 mixture of *cis*/*trans* compounds resulted (Scheme 1). While isomerisation of the *trans*-product after the sodium methanolate deacetylation step (**7c**) might be explained by a base catalyzed mechanism,[14] the *cis*/*trans* mixture in the metathesis product **5c** is probably caused by photoisomerisation.

In case of the methoxy-substituted styrene, the conversion was incomplete, however, only the *trans* configured product **5b** could be obtained. A proposed lower reactivity of the intermediate *p*-methoxybenzylidene ruthenium complex in comparison to the unsubstituted benzylidene complex is reported by Grubbs *et al.*,[13,15] so it is possible that decomposition of the catalyst occurred before the reaction was completed.

Deprotection of **5a–c** in two steps led straight forwardly to the *C*-sialoside ligands **9a–c**. The lower yields in case of the nitro-compounds might be due to degradation processes, indicated by a deepening in the original yellow colour of these compounds over the time.

The recently reported hydrogenated metathesis products of α- an β-configured *C*-sialosides **10** and **11** (Scheme 2)[11] were also deprotected to give the ligands **12** and **13**.

The deacetylated nitro-substituted metathesis product **7c** could easily be hydrogenated to give the saturated amino-substituted *C*-sialoside **14** (Scheme 3).

Deprotection to *C*-sialoside ligand **15** was conducted with triethylamine in water to avoid loss of product during the ion exchange neutralisation step necessary in sodium hydroxide saponification.

Acetylation of **14** gave the *N*-acetyl derivative **16**. In this case the deprotection steps succeeded in average yields partly due to slight base lability of the aromatic *N*-acetyl group and partly due to the pronounced hydrophilicity of the compounds **16**, **17** and **18** during chromatography.

Reductive benzylation of the glycerol side-chain. The 9-hydroxyl group of sialic acid is supposed to bind to the indole nitrogen of Trp_{120} as supported by data of the crystal structure of TcTS (see Fig. 2).[9] Therefore, an additional aromatic ring in this position was supposed to be advantageous for TcTS binding of these ligands.

Application of a method for 9-*O*-benzylation of sialic acid derivatives recently reported by Ernst *et al.*[16] to compound **6** gave 8,9-*O*-benzylidene acetal **19** (1 : 1 mixture of diastereomers) as well as 7,9-*O*-benzylidene acetal **20** as a single species displaying an all equatorial six-membered chair as resolved by two dimensional nmr spectroscopy (Scheme 4). Interestingly, compound **20** did

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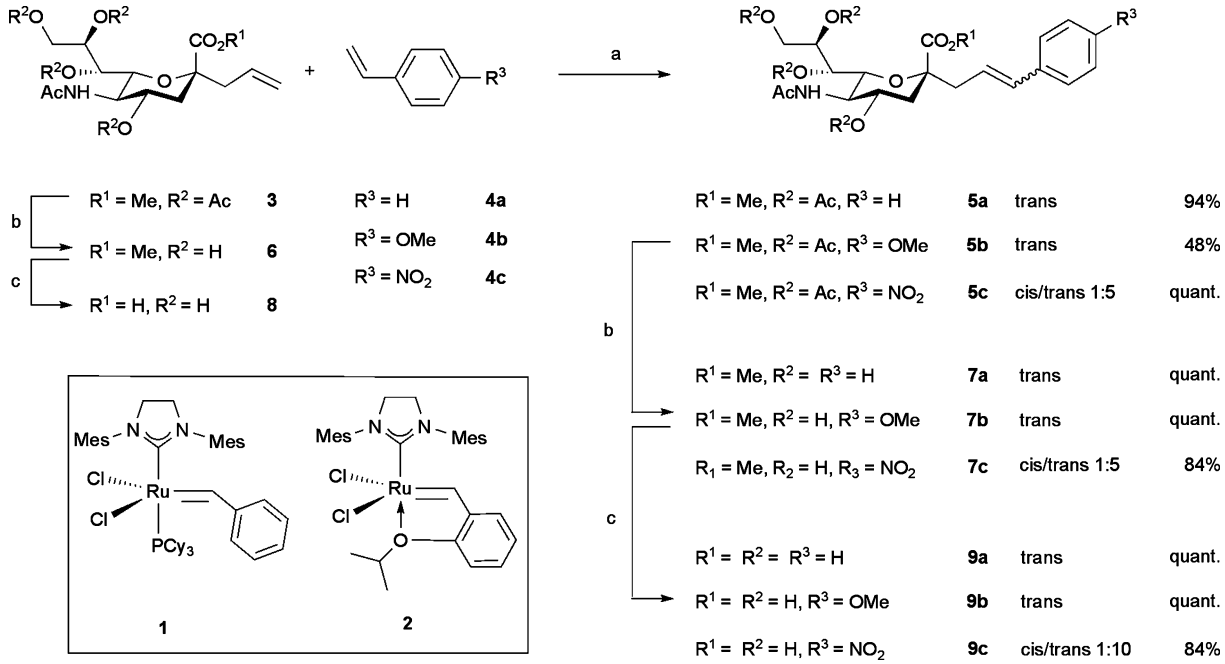
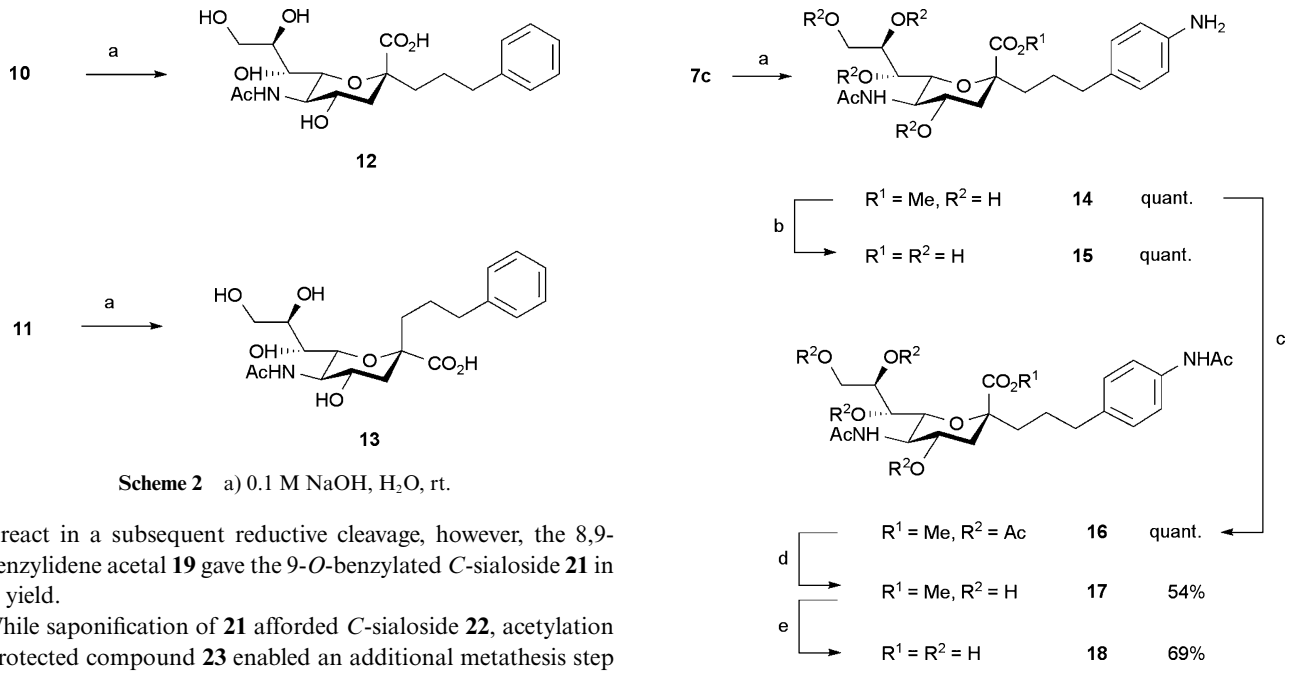


Scheme 1 a) Ruthenium catalyst **1** or **2**, CH_2Cl_2, reflux; b) NaOMe, MeOH, rt; c) 0.1 M NaOH, H_2O, rt.



Scheme 2 a) 0.1 M NaOH, H_2O, rt.

Scheme 3 a) H_2, Pd/C, MeOH, rt; b) Et_3N, H_2O, rt; c) Ac_2O, pyridine, rt; d) NaOMe, MeOH, rt; e) 0.1 M NaOH, H_2O, rt.

not react in a subsequent reductive cleavage, however, the 8,9-*O*-benzylidene acetal **19** gave the 9-*O*-benzylated *C*-sialoside **21** in 51% yield.

While saponification of **21** afforded *C*-sialoside **22**, acetylation to protected compound **23** enabled an additional metathesis step (Scheme 5), leading to an interesting molecule with two aromatic rings in terminal positions. The yield in the cross metathesis reaction with styrene was satisfactory in comparison to non-benzylated compounds.[11]

Introducing the carboxylate in the 4-position. The presence of several arginine residues in the active site leads to the idea to place an additional carboxylate residue as a useful substituent in the Neu5Ac ring system. Aside from the important binding of the Neu5Ac carboxylate to arginine, there are two further arginine residues located near the 4-hydroxyl group. One of these arginines (Arg_{53}) is supposed to interact with the 4-hydroxyl group *via* hydrogen bonding (see Fig. 3).[9]

Utilising the method of Zbiral[17] for compound **7a** the glycerol side chain was protected by an isopropylidene group to yield **27**. The subsequent oxidation by pyridinium chlorochromate lead to the 4-ketone **28** in satisfactory yield, which by Wittig reaction gave the unsaturated ethyl ester **29** in 45% yield (Scheme 6). In the subsequent hydrogenation step only the product with equatorial orientation of the C4 substituent **30** was formed, and the final two step deprotection procedure gave *C*-sialoside **32** bearing two carboxyl groups.

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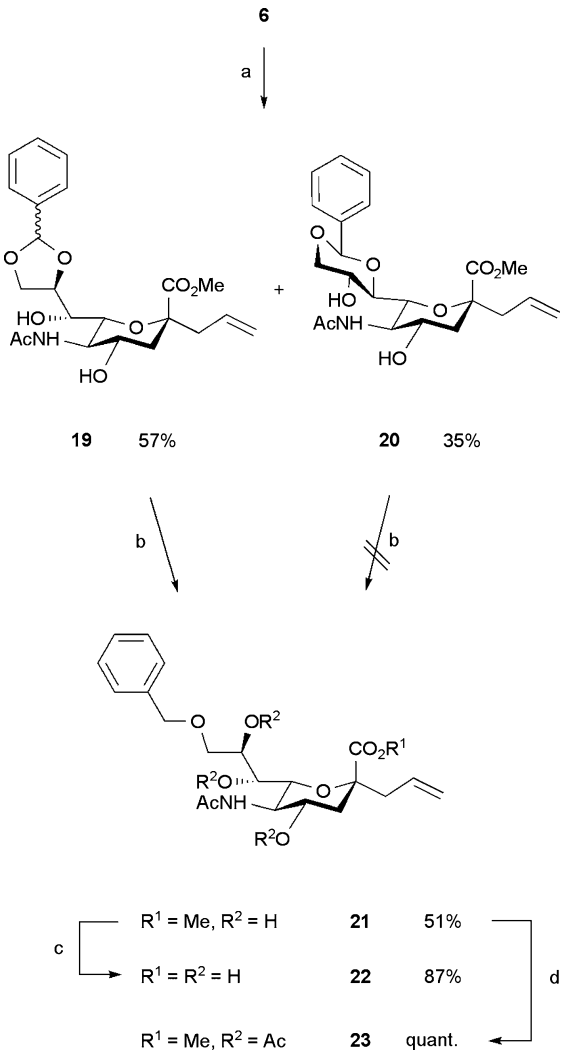
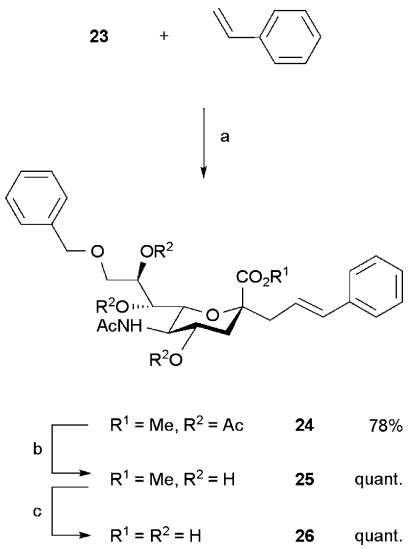
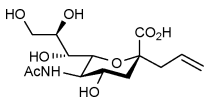
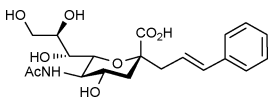
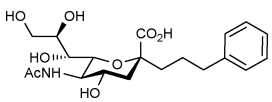
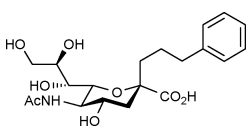


Scheme 4 a) BADMA, pTSA, MeCN, 0 °C; b) BH_3–Me_3N, $AlCl_3$, THF, H_2O, rt; c) 0.1 M NaOH, H_2O, rt; d) Ac_2O, pyridine.



Scheme 5 a) catalyst **1**, CH_2Cl_2, reflux; b) NaOMe, MeOH, rt; c) 0.1 M NaOH, H_2O, rt.

Table 1 Influence of aromatic residue and configuration on binding

	Compound	K_D (mM)
8		n. d.[a]
9a		3.6 ± 0.6
12		0.16 ± 0.02
13		n. d.[a]

[a] The K_D values obtained were in the molar range.

Surface plasmon resonance affinity measurements

Recombinant *trans*-sialidase[18] was immobilised on a commercially available CM5 sensor chip (BIACORE) using the amino coupling method as described by the manufacturer. The affinity measurements were performed on a BIACORE T100 instrument with Tris-HCl (pH = 7.5, 100 mM) as running buffer using 7–10 different dilutions of synthetic ligands up to 5 mM. The K_D-values were calculated using OriginPro 7.5 and the BIACORE evaluation software.

The K_D values obtained of these ligands vary from 0.16 to 14 mM and thus are in the range of common monovalent carbohydrate protein interactions.[19,20]

The results in Table 1 clearly indicate the impact of an aromatic group attached to the ligand considered to mimic the sugar residue of the natural substrate bound between two aromatic amino acid side chains (Tyr_{119} and Trp_{312}, see Fig. 1). A missing aromatic residue as in compound **8** as well as compounds with a β-configured residue as in **13** clearly show no binding, which indicate the specific interactions in the active site of TcTS.

Hydrogenation of the double bond increases affinity for TcTS (compound **12** in comparison to compound **9a**), probably due to an increased flexibility of the phenylpropyl residue. Compound **12** shows the best K_D value (0.16 mM) in this work, and its SPR response curves and the resulting curve fit is shown as an example in Fig. 5.

A further improvement of binding by *para*-substituents attached to the aromatic ring was hitherto not observed (Table 2).

The roughly doubled K_D of the nitro-compound **9c** measured as a mixture of *cis*/*trans* isomers could be interpreted as a significantly reduced binding of the *cis*-isomer. However, since the proportion of the *cis*-isomer is low (*ca.* 10%), the binding of the *trans*-isomer is likely to be the main cause for the overall K_D value of **9c**.

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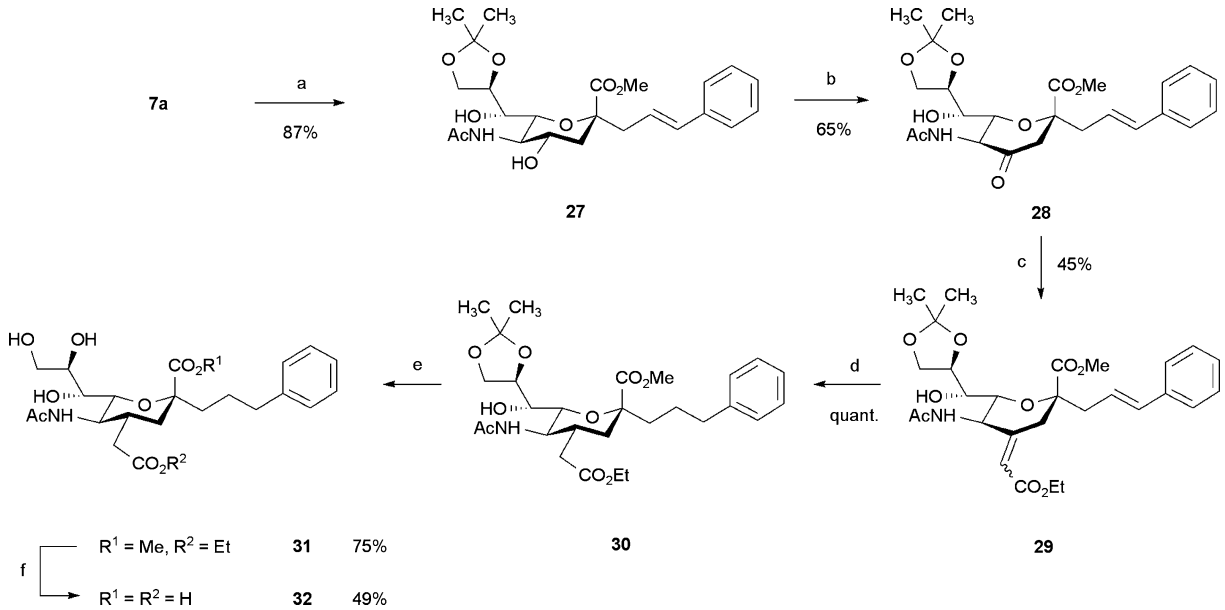


Scheme 6 a) 2,2-Dimethoxy propane, H^+ (ion exchange), acetone, rt; b) PCC, mol sieve 4 Å, CH_2Cl_2, rt; c) $Ph_3P{=}CHCO_2Et$, THF, rt; d) H_2, Pd/C, MeOH, rt; e) H^+ (ion exchange), H_2O, 60 °C; f) 0.1 M NaOH, H_2O, rt.

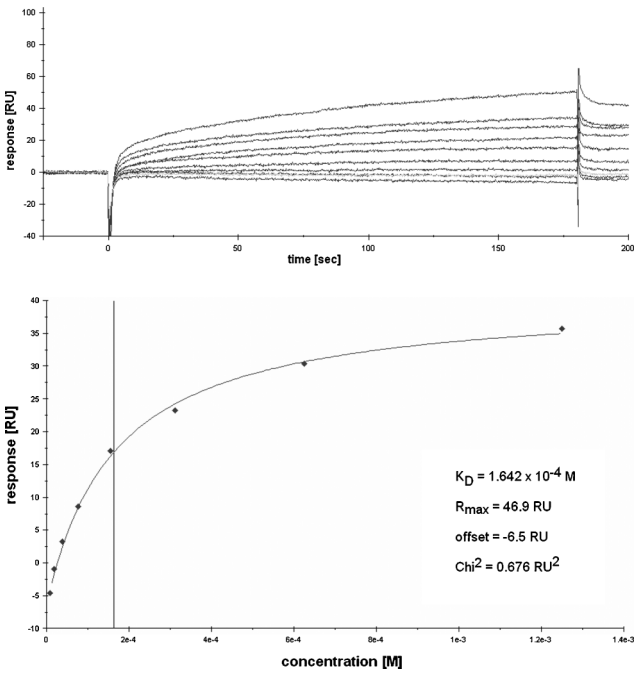
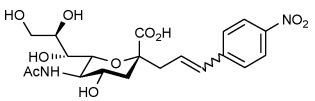
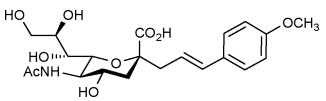
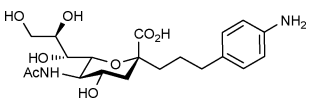
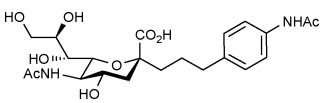


Fig. 5 Response curves and curve fit for ligand **12**.

Table 2 Aromatic *C*-sialosides with substituents

	Compound	K_D (mM)
9c		11.5 ± 1.9^a
9b		5.0 ± 3.0
15		0.35 ± 0.12^b
18		3.9 ± 1.4^b

[a] *cis*/*trans*-mixture. [b] Errors calculated on basis of average percental aberration of K_D values.

The hydrophilic amino group in compound **15** with a K_D value similar to compound **12** seems to eliminate the possibility of mere hydrophobic interaction in the binding of aromatic *C*-sialosides to TcTS.

The trend for better binding associated with greater flexibility is obvious with the K_D values of **15** and **18**.

Ligand binding is also enabled by an aromatic group connected to the glycerol side chain as in compound **23** (Table 3). Very likely this binding event also occurs between Tyr_{119} and Trp_{312} (see Fig. 1) as suggested by recent results of Withers *et al.*,[21] who observed an aromatic group attached to the glycerol side chain to be located in the acceptor binding site as shown by X-ray crystal structure.

The possibility of an additional binding to Trp_{120} as mentioned above is eliminated by the fact that two aromatic residues in the same molecule do not improve binding as demonstrated with compound **26**. Based on the results of Withers *et al.*[21] it can be assumed that steric hindrance between two aromatic rings in the acceptor binding site is responsible for decrease in affinity of compound **26**.

Interestingly, the active site tolerates the additional carboxylate in compound **32**, although no stronger binding results from this synthetic variation. Perhaps stronger binding due negatively charged carboxylate is counteracted by steric demands.

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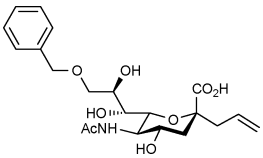
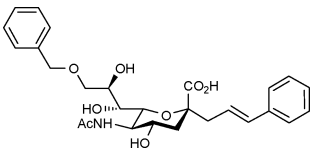
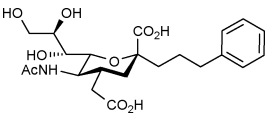
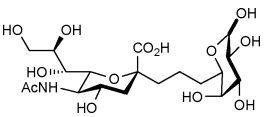
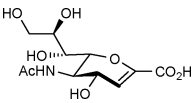
Table 3 Other *C*-sialoside derivatives

	Compound	K_D (mM)
22		0.37 ± 0.07
26		2.0 ± 0.3
32		3.5 ± 0.4
33		14 ± 5
34		0.65 ± 0.85

The chain elongated *C*-analogue of α(2–6)-sialyl galactose **33**[11] does also not show any increase of affinity, which is probably caused by mimicking the "wrong" α(2–6)-linkage which is not recognised by this enzyme. A corresponding decrease in binding by an α(2–6)-sialyl galactose analogue containing a phosphonate group in comparison to its α(2–3)-isomer was observed by Streicher *et al.*[8]

As reference the glycal of Neu5Ac (Neu5Ac2en, compound **34**) was tested under similar conditions. Neu5Ac2en is known as an inhibitor of viral and bacterial sialidases,[22] but shows only weak inhibition towards TcTS.[23,24] Since TcTS displays a mechanism similar to that of sialidases,[9,25] Neu5Ac2en can be considered as transition state analogue for TcTS and should naturally show affinity to this enzyme. Interestingly, the K_D value of **34** is in the submillimolar range, while a poor inhibitory constant of 12.3 mM is reported in the literature.[5] Since it is generally difficult to compare K_D and K_i values, this suggests that further studies are needed regarding these compounds.

NMR based inhibition assay

Ligand **12** showed the highest affinity towards TcTS in the SPR experiments (K_D = 0.16 mM) and was therefore subjected to a preliminary inhibition assay based on the TcTS catalyzed sialylation of methyl allolactoside employing *p*NP-sialoside as donor substrate in D_2O. Transfer rates were determined by proton NMR.[18] As shown in Fig. 6 we observed a considerable decrease in *trans*-sialidase activity at a ligand concentration of 1 mM.

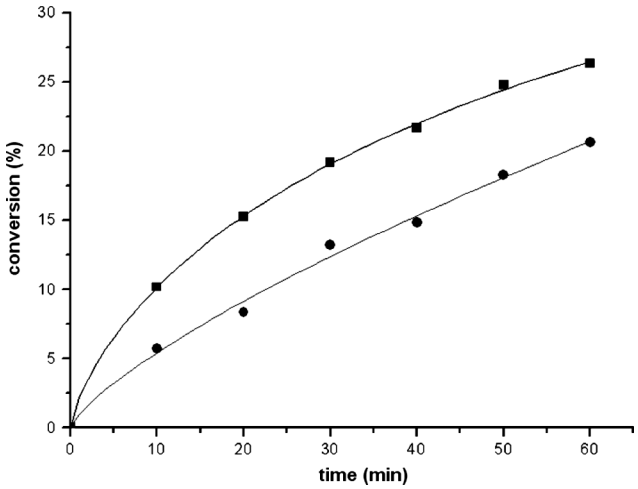


Fig. 6 Transfer rates measured by NMR. Compound **12** leads to a decrease in enzyme activity at a concentration of 1 mM.

Conclusions

Cross metathesis turned out to be a useful tool in synthesising a series of differently substituted aromatic *C*-sialosides. This method, once established, allows to use various substituted styrenes, thus providing the means to fine-tune electronic properties of ligands. This approach should prove generally useful in the field of ligand synthesis regarding studies of carbohydrate protein interactions.

The K_D values obtained by SPR and discussed above provide experimental evidence to qualitative considerations deduced by inspection of the crystal structure of TcTS in complex with sialyl lactose.[9] The most convincing result is the considerable influence on ligand binding by addition of an aromatic ring to the α-*C*-sialoside, which clearly indicates the importance of mimicking the acceptor molecule in TcTS ligand design. Additionally it was shown that two aromatic rings as in compound **26** are sterically too demanding for the acceptor binding site between Tyr_{119} and Trp_{312}. Compound **12** with the best affinity (K_D = 0.16 mM) represents a novel class of *C*-glycoside ligands for TcTS. Such *C*-glycosides do not only provide hydrolytic stability but also allow for versatile derivatisation in the development of stronger binding ligands.

A preliminary NMR based inhibition assay demonstrated that inhibition of TcTS by this type of compounds is possible. Selective derivatisation of **12** should permit the synthesis of more effective inhibitors for TcTS.

Experimental

General methods

Commercially available starting materials were used without further purification. Solvents were dried according to standard methods. TLC was performed on precoated aluminium plates (Silica Gel 60 F254, Merck 5554) employing UV-absorption and charring with 10% H_2SO_4 in ethanol for visualisation. For column chromatography Silica Gel 60, 230–400 mesh, 40–63 μm (Merck) was used.

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^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AMX-400 (400.25 MHz for ^{1}H, 100.65 MHz for ^{13}C), Bruker AV-400 (400.25 MHz for ^{1}H, 100.65 MHz for ^{13}C) and on Bruker DRX-500 (500.13 MHz for ^{1}H, 125.77 MHz for ^{13}C) at 300 K. Chemical shifts were calibrated to solvent residual peaks.[26] The signals were assigned by HH-COSY, HSQC, HMBC and if necessary NOESY experiments. *J* values are given in Hz.

Optical rotations were measured using a Perkin Elmer 241 (546 nm) or a Krüss Optronic P8000 (589 nm) at 20 °C; $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}.

Mass spectra were recorded on a Bruker Biflex III in positive reflector mode (MALDI-TOF), on a VG Analytical VG/70-250 F (FAB) and on a Thermo Finnigan MAT 95 XL mass spectrometer (ESI).

Allyl *C*-sialosides **3**, **6**, **8**[10] and substituted styrenes **4b** and **4c**[12] were synthesised as described and the data were consistent with those published. The syntheses of *C*-sialosides **5a**, **10**, **11** and **33** were published before in a short communication.[11]

The synthetic routes leading to compounds **9b**, **22** and **32** are shown in the following section as examples for the three derivatisation methods employed in this work. The complete experimental data for all new compounds can be found in the ESI.†

Syntheses

General procedure for cross metathesis with styrenes (GP1). The allyl *C*-sialoside was dissolved under nitrogen in anhydrous dichloromethane to yield a 0.002 M solution and the styrene (8–10-fold excess) was added. After addition of the ruthenium catalyst, the reaction was stirred under reflux for 24 h. The removal of the solvent *in vacuo* and column chromatography on silica gel (toluene/acetone or pure ethyl acetate) yielded the product.

General procedure for acetylation (GP2). The compound was dissolved in pyridine and an excess of acetic anhydride was added. The solution was stirred at room temperature until TLC indicated complete consumption of starting material. The solvent was removed *in vacuo* and the residue was co-evaporated with toluene before column chromatography (silica gel, toluene/acetone or pure ethyl acetate).

General procedure for deacetylation (GP3). The compound was dissolved in dry methanol and sodium methoxide (1 molar) in dry methanol was added drop wise until a pH of 9–10. The reaction was stirred at room temperature until TLC showed complete deacetylation. After neutralisation with acidic ion exchanger and filtration the solvent was removed *in vacuo*. Column chromatography on silica gel (dichloromethane/methanol) yielded the product.

General procedure for ester saponification (GP4). The ester was dissolved in aqueous sodium hydroxide (0.1 M) and stirred at room temperature until TLC showed complete conversion. After neutralisation with acidic ion exchanger and filtration the material was freeze dried.

General procedure for catalytic hydrogenation (GP5). The unsaturated compound was dissolved in methanol under nitrogen. After adding a catalytic amount of palladium on charcoal (5%, wet) the reaction vessel was carefully evaporated and flushed with hydrogen. The reaction was stirred under hydrogen at room temperature until TLC showed complete conversion of the starting material. Column chromatography on silica gel (dichloromethane/methanol) yielded the product.

(*E*)-Methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-2-*C*-(3-(4-methoxyphenyl)-prop-2-enyl)-D-*erythro*-L-*manno*-nononate (5b). **a**) Compound **3** (240 mg, 466 µmol) was reacted with **4b** (540 mg, 4.02 mmol) according to **GP1** using catalyst **2** (25 mg, 40 µmol) to give **5b** in a mixture with starting material **3** (136 mg, 47%; calculated from NMR) as a colourless oil;

b) **7b** (13 mg, 29 µmol) was acetylated according to **GP2** to give **5b** (18 mg, 100%) as a colourless oil;

$[\alpha]_D^{25}$ −13.8 (*c* 1 in $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 7.31 (2 H, d, *J* 8.7, arom. H), 6.84 (2 H, d, *J* 8.7, arom. H), 6.31 (1 H, d, *J* 15.8, -CH=C*H*–Ph), 6.00 (1 H, ddd, *J* 15.8, 8.0 and 6.8, -CH_2–C*H*=), 5.40 (1 H, ddd, $J_{8,7}$ 6.9, $J_{8,9b}$ 6.0, $J_{8,9a}$ 2.6, H-8), 5.34 (1 H, dd, $J_{7,8}$ 6.9, $J_{7,6}$ 1.1, H-7), 5.17 (1 H, d, $J_{NH,5}$ 9.6, N*H*), 4.84 (1 H, ddd, $J_{4,3ax}$ 11.7, $J_{4,5}$ 10.0, $J_{4,3eq}$ 4.6, H-4), 4.42 (1 H, dd, $J_{9a,9b}$ 12.4, $J_{9a,8}$ 2.6, H-9a), 4.15 (1 H, dd, $J_{9b,9a}$ 12.4, $J_{9b,8}$ 6.0, H-9b), 4.05–4.00 (2 H, m, H-5, H-6), 3.80 (3 H, s, arom. OCH_3), 3.70 (3 H, s, OCH_3), 2.65–2.50 (2 H, m, -CH_2–CH=), 2.51 (1 H, dd, $J_{3eq,3ax}$ 12.9, $J_{3eq,4}$ 4.6, H-3$_{eq}$), 2.12, 2.11, 2.02, 2.01, (each 3 H, 4x s, 4x AcCH_3), 1.87 (3 H, s, NHAcCH_3), 1.83 (1 H, dd, $J_{3ax,3eq}$ 12.9, $J_{3ax,4}$ 11.7, H-3$_{ax}$); δ_C (125 MHz, $CDCl_3$) 171.77 (*C*O_2CH_3), 171.23, 170.86, 170.43, 170.35, 170.24 (5x Ac*C*=O), 159.26 (arom. C), 133.63 (-CH=*C*H–Ph), 129.96 (arom. C), 127.69 (arom. C), 120.48 (-CH_2–*C*H=), 114.04 (arom. C), 80.97 (C-2), 73.66 (C-6), 70.37 (C-4), 69.87 (C-8), 68.08 (C-7), 62.55 (C-9), 55.43 (arom. O*C*H_3), 52.52 (O*C*H_3), 49.81 (C-5), 43.74 (-*C*H_2-CH=), 37.52 (C-3), 23.35 (NHAc*C*H_3), 21.26, 21.04 (2 × Ac*C*H_3), 20.94 (2 × Ac*C*H_3); HRMS (FAB): *m*/*z* calcd. for $[M+H]^+$: 622.249966; found: 622.250854.

(*E*)-Methyl-5-acetamido-2,6-anhydro-3,5-dideoxy-2-*C*-(3-(4-methoxyphenyl)-prop-2-enyl)-D-*erythro*-L-*manno*-nononate (7b). Compound **5b** (136 mg, 0.230 mmol) was deacetylated according to **GP3** to give **7b** (97 mg, 100%) as a colourless oil; $[\alpha]_D^{25}$ +4.2 (*c* 1 in MeOH); δ_H (400 MHz, $[D_4]$MeOH) 7.28 (2 H, d, *J* 8.8, arom. H), 6.84 (2 H, d, *J* 8.8, arom. H), 6.38 (1 H, d, *J* 15.8, -CH=C*H*–Ph), 6.05 (1 H, ddd, *J* 15.8, 7.5 and 7.5, -CH_2–C*H*=), 3.89 (1 H, ddd, $J_{8,7}$ 8.9, $J_{8,9b}$ 5.7, $J_{8,9a}$ 2.8, H-8), 3.85 (1 H, dd, $J_{9a,9b}$ 11.3, $J_{9a,8}$ 2.8, H-9a), 3.77 (3 H, s, arom. OCH_3), 3.75 (3 H, s, OCH_3), 3.73 (1 H, dd, $J_{5,6}$ 10.2, $J_{5,4}$ 10.1, H-5), 3.68-3.60 (2 H, m, H-4, H-9b), 3.52 (1 H, dd, $J_{6,5}$ 10.2, $J_{6,7}$ 1.6, H-6), 3.51 (1 H, dd, $J_{7,8}$ 8.9, $J_{7,6}$ 1.6, H-7), 2.65–2.59 (3 H, m, -CH_2–CH= and H-3$_{eq}$), 2.00 (3 H, s, AcCH_3), 1.65 (1 H, dd, $J_{3ax,3eq}$ 13.1, $J_{3ax,4}$ 11.5, H-3$_{ax}$); δ_C (100 MHz, $[D_4]$MeOH) 175.24 (2 × *C*=O), 160.72 (arom. C), 134.74 (-CH=*C*H–Ph), 131.34 (arom. C), 128.47 (arom. C), 121.66 (-CH_2–*C*H=), 114.96 (arom. C), 82.19 (C-2), 75.95 (C-6), 72.91 (C-8), 70.26 (C-7), 69.14 (C-4), 64.67 (C-9), 55.71 (arom. O*C*H_3), 54.19 (C-5), 53.04 (O*C*H_3), 44.90 (-*C*H_2–CH=), 41.46 (C-3), 22.64 (Ac*C*H_3); HRMS (ESI): *m*/*z* calcd. for $[M+Na]^+$: 476.1891; found: 476.1897.

(*E*)-5-Acetamido-2,6-anhydro-3,5-dideoxy-2-*C*-(3-(4-methoxyphenyl)-prop-2-enyl)-D-*erythro*-L-*manno*-nononic acid (9b). Compound **7b** (12 mg, 26 µmol) was saponified according to **GP4** to give **9b** (12 mg, 100%) as a yellow amorphous solid; $[\alpha]_D^{25}$ +7.0 (*c* 0.5 in MeOH); δ_H (400 MHz, D_2O) 7.41 (2 H, d, *J* 8.8, arom. H),

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6.97 (2 H, d, *J* 8.8, arom. H), 6.49 (1 H, d, *J* 15.9, -CH=C*H*–Ph), 6.12 (1 H, ddd, *J* 15.9 and 2 × 7.5, -CH_2–C*H*=), 3.89 (1 H, ddd, $J_{8,7}$ 8.6, $J_{8,9b}$ 6.2, $J_{8,9a}$ 2.7, H-8), 3.90–3.84 (1 H, m, H-9a), 3.83 (3 H, s, arom. OCH_3), 3.83–3.78 (1 H, m, H-5), 3.74 (1 H, ddd, $J_{4,3ax}$ 11.7, $J_{4,5}$ 10.0, $J_{4,3eq}$ 4.6, H-4), 3.66 (1 H, dd, $J_{6,5}$ 10.2, $J_{6,7}$ 1.5, H-6), 3.66–3.62 (1 H, m, H-9b), 3.59 (1 H, dd, $J_{7,8}$ 8.6, $J_{7,6}$ 1.5, H-7), 2.66 (1 H, dd, $J_{3eq,3ax}$ 13.0, $J_{3eq,4}$ 4.6, H-3_{eq}), 2.65–2.64 (2 H, m, -CH_2–CH=), 2.05 (3 H, s, AcCH_3), 1.69 (1 H, dd, $J_{3ax,3eq}$ 13.0, $J_{3ax,4}$ 11.7, H-3_{ax}); δ_C (100 MHz, D_2O) 176.75 (*C*=O), 132.95 (-CH=*C*H–Ph), 130.37 (arom. C), 127.58 (arom. C), 121.67 (-CH_2–*C*H=), 114.32 (arom. C), 81.01 (C-2), 73.76 (C-6), 71.90 (C-8), 68.38, 68.35 (C-4, C-7), 62.80 (C-9), 55.43 (arom. OCH_3), 52.24 (C-5), 43.05 (-*C*H_2–CH=), 39.71 (C-3), 22.06 (AcCH_3); MS (MALDI-TOF): *m*/*z* = 440.5 ([M+H]$^+$), 462.4 ([M+Na]$^+$), 478.4 ([M+K]$^+$); MS (FAB): *m*/*z* = 462.3 ([M+Na]$^+$), 478.3 ([M+K]$^+$).

Methyl-5-acetamido-2,6-anhydro-8,9-*O*-benzyliden-3,5-dideoxy-2-*C*-(prop-2-enyl)-D-*erythro*-L-*manno*-nononate (19) and methyl-5-acetamido-2,6-anhydro-7,9-*O*-benzyliden-3,5-dideoxy-2-*C*-(prop-2-enyl)-D-*erythro*-L-*manno*-nononate (20). Compound **6** (200 mg, 0.576 mmol) and benzaldehyde dimethyl acetal (173 µL, 1.15 mmol) were dissolved in dry acetonitrile. The solution was cooled to 0 °C and a catalytic amount (8 mg, 0.04 mmol) of *p*-toluenesulfonic acid monohydrate was added. The reaction was stirred at room temperature until TLC showed complete consumption of the starting material. After adding a few drops of triethyl amine the solvent was removed *in vacuo* and the products were separated by column chromatography (dichloromethane/methanol); **19** (143 mg, 57%, 1 : 1 mixture of diastereomers): mp 112 °C; $[\alpha]_D^{25}$ +83 (*c* 0.1 in CH_2Cl_2); δ_H (500 MHz, [D_4]MeOH) 7.50–7.46 (2 H, m, arom. H), 7.38–7.35 (3 H, m, arom. H), 5.87 (0.5 H, s, PhC*H*^{I}), 5.85–5.74 (2 H, m, C*H*=CH_2^I, C*H*=CH_2^{II}), 5.76 (1 H, s, PhC*H*^{II}), 5.12–5.06 (4 H, m, CH=CH_2^I, CH=CH_2^{II}), 4.40–4.34 (2 H, m, H-8), 4.27 (1 H, dd, $J_{9a,9b}$ 8.5, $J_{9a,8}$ 6.7, H-9a^I), 4.24 (1 H, dd, $J_{9a,9b}$ 8.3, $J_{9a,8}$ 5.2, H-9a^{II}), 4.11 (1 H, dd, $J_{9b,9a}$ 8.5, $J_{9b,8}$ 7.6, H-9b^I), 4.09 (1 H, dd, $J_{9b,9a}$ 8.3, $J_{9b,8}$ 7.3, H-9b^{II}), 3.82 (1 H, dd, $J_{7,8}$ 5.2, $J_{7,6}$ 1.1, H-7^I), 3.75 (1 H, dd, $J_{5,4}$ 10.2, $J_{5,6}$ 10.2, H-5^I), 3.74 (1 H, dd, $J_{5,4}$ 10.2, $J_{5,6}$ 10.2, H-5^{II}), 3.69–3.59 (3 H, m, H-7^{II}, H-4^I, H-4^{II}), 3.66 (3 H, s, OCH_3), 3.65 (3 H, s, OCH_3), 3.50 (1 H, dd, $J_{6,5}$ 10.2, $J_{6,7}$ 1.1, H-6^{II}), 3.47 (1 H, dd, $J_{6,5}$ 10.2, $J_{6,7}$ 1.1, H-6^I), 2.56–2.44 (6 H, m, CH_2^I, CH_2^{II}, H-3_{eq}^I, H-3_{eq}^{II}), 2.01 (3 H, s, AcCH_3), 1.98 (3 H, s, AcCH_3), 1.54 (1 H, dd, $J_{3ax,3eq}$ 12.8, $J_{3ax,4}$ 11.7, H-3_{ax}), 1.53 (1 H, dd, $J_{3ax,3eq}$ 12.8, $J_{3ax,4}$ 11.7, H-3_{ax}); δ_C (125 MHz, [D_4]MeOH) 139.59 (arom. C), 133.21, 133.16 (-*C*H=CH_2), 130.31, 130.14, 129.23, 127.97, 127.68 (arom. C), 119.26, 119.17 (-CH=*C*H_2), 105.08 (Ph*C*H^{II}), 104.84 (Ph*C*H^I), 81.99, 81.88 (C-2), 78.11, 77.92 (C-8), 76.72, 76.31 (C-6), 70.93, 70.89 (C-7), 69.07, 68.92 (C-4), 68.59, 68.48 (C-9), 54.11 (C-5), 52.46, 52.43 (OCH_3), 45.64, 45.61 (*C*H_2), 41.27 (C-3), 22.72 (AcCH_3); HRMS (FAB): *m*/*z* calcd. for [M+H]$^+$: 436.197142; found: 436.197968; **20** (88 mg, 35%): mp 83–84 °C; $[\alpha]_D^{25}$ −21.4 (*c* 0.5 in MeOH); δ_H (500 MHz, [D_4]MeOH) 7.59–7.56 (2 H, m, arom. H), 7.37–7.30 (3 H, m, arom. H), 5.88–5.75 (1 H, m, C*H*=CH_2), 5.43 (1 H, s, PhC*H*), 5.07–5.03 (2 H, m, CH=CH_2), 4.27 (1 H, dd, $J_{9a,9b}$ 10.6, $J_{9a,8}$ 5.4, H-9a), 4.04 (1 H, ddd, $J_{8,9b}$ 10.0, $J_{8,7}$ 9.7, $J_{8,9a}$ 5.4, H-8), 4.02 (1 H, dd, $J_{5,6}$ 10.5, $J_{5,4}$ 10.3, H-5), 3.87 (1 H, dd, $J_{6,5}$ 10.5, $J_{6,7}$ 1.1, H-6), 3.74 (3 H, s, OCH_3), 3.60 (1 H, ddd, $J_{4,3ax}$ 11.9, $J_{4,5}$ 10.3, $J_{4,3eq}$ 4.7, H-4), 3.56 (1 H, dd, $J_{9b,9a}$ 10.6, $J_{9b,8}$ 10.0, H-9b), 3.55 (1 H, dd, $J_{7,8}$ 9.7, $J_{7,6}$ 1.1, H-7), 2.54–2.42 (3 H, m, CH_2, H-3_{eq}), 1.99 (3 H, s, AcCH_3), 1.54 (1 H, dd, $J_{3ax,3eq}$ 12.8, $J_{3ax,4}$ 11.9, H-3_{ax}); δ_C (125 MHz, [D_4]MeOH) 133.41 (-*C*H=CH_2), 129.50, 128.84, 127.50 (arom. C), 118.89 (-CH=*C*H_2), 102.24 (Ph*C*H), 81.77 (C-2), 80.78 (C-7), 73.16 (C-6), 72.62 (C-9), 69.76 (C-4), 61.36 (C-8), 52.75 (C-5), 52.55 (OCH_3), 45.58 (*C*H_2), 41.11 (C-3), 22.93 (AcCH_3); HRMS (FAB): *m*/*z* calcd. for [M+H]$^+$: 436.197142; found: 436.197968.

Methyl-5-acetamido-2,6-anhydro-9-*O*-benzyl-3,5-dideoxy-2-*C*-(prop-2-enyl)-D-*erythro*-L-*manno*-nononate (21). Compound **19** (160 mg, 367 µmol), trimethyl amine borane adduct (0.11 g, 1.5 mmol) and aluminium chloride (294 mg, 2.20 mmol) were dissolved in 8.4 mL dry tetrahydrofuran. After stirring for 5 min water (9.9 µL, 0.55 mmol) was added and the reaction was stirred at room temperature for 4 h. After TLC showed complete consumption of starting material the reaction was stopped by adding water (4.2 mL) and 0.1 molar HCl (4.2 mL). The solution was diluted with dichloromethane (42 mL) and washed twice with 5% sodium bicarbonate solution (2 × 20 mL) and with water (20 mL). The organic phase was dried over sodium sulphate, filtrated and concentrated *in vacuo*. The residue was purified by column chromatography (dichloromethane/methanol) to give **21** (82 mg, 51%) as a white solid; mp 101 °C; $[\alpha]_D^{25}$ −104 (*c* 0.2 in CH_2Cl_2); δ_H (500 MHz, [D_4]MeOH) 7.38–7.31 (5 H, m, arom. H), 5.84–5.74 (1 H, m, C*H*=CH_2), 5.11–5.06 (2 H, m, CH=CH_2), 4.59–4.57 (2 H, m, PhCH_2), 3.97 (1 H, ddd, $J_{8,7}$ 8.8, $J_{8,9b}$ 5.7, $J_{8,9a}$ 2.3, H-8), 3.78 (1 H, dd, $J_{9a,9b}$ 10.3, $J_{9a,8}$ 2.3, H-9a), 3.75 (3 H, s, OCH_3), 3.70 (1 H, dd, $J_{5,4}$ 10.2, $J_{5,6}$ 10.2, H-5), 3.65–3.58 (1 H, m, H-4), 3.62 (1 H, dd, $J_{9b,9a}$ 10.3, $J_{9b,8}$ 5.7, H-9b), 3.55 (1 H, dd, $J_{7,8}$ 8.8, $J_{7,6}$ 1.4, H-7), 3.51 (1 H, dd, $J_{6,5}$ 10.2, $J_{6,7}$ 1.4, H-6), 2.57 (1 H, dd, $J_{3eq,3ax}$ 13.2, $J_{3eq,4}$ 4.6, H-3_{eq}), 2.54–2.44 (2 H, m, CH_2), 1.98 (3 H, s, AcCH_3), 1.45 (1 H, dd, $J_{3ax,3eq}$ 13.2, $J_{3ax,4}$ 11.4, H-3_{ax}); δ_C (100 MHz, [D_4]MeOH) 175.22 (C-1), 139.85 (arom C), 132.94 (-*C*H=CH_2), 129.33 (arom. C), 128.84 (arom. C), 128.59 (arom. C), 119.37 (-CH=*C*H_2), 81.75 (C-2), 75.78 (C-6), 74.41 (Ph*C*H_2), 72.90 (C-9), 71.82 (C-8), 70.16 (C-7), 69.04 (C-4), 54.17 (C-5), 52.95 (OCH_3), 45.71 (*C*H_2), 41.45 (C-3), 22.63 (AcCH_3); HRMS (FAB): *m*/*z* calcd. for [M+H]$^+$: 438.212792; found: 438.211823.

5-Acetamido-2,6-anhydro-9-*O*-benzyl-3,5-dideoxy-2-*C*-(prop-2-enyl)-D-*erythro*-L-*manno*-nononic acid (22). Compound **21** (62 mg, 0.14 mmol) was saponified according to **GP4** to give **22** (60 mg, 100%) as a white solid; mp 147 °C; $[\alpha]_D^{25}$ −22 (*c* 0.1 in H_2O); δ_H (500 MHz, [D_4]MeOH) 7.37–7.23 (5 H, m, arom. H), 5.94–5.85 (1 H, m, C*H*=CH_2), 5.08–4.99 (2 H, m, CH=CH_2), 4.59 (1 H, d, *J* 12.3, PhCH_2a), 4.54 (1 H, d, *J* 12.3, PhCH_2b), 3.99 (1 H, ddd, $J_{8,7}$ 8.8, $J_{8,9b}$ 5.7, $J_{8,9a}$ 2.2, H-8), 3.76 (1 H, dd, $J_{9a,9b}$ 10.4, $J_{9a,8}$ 2.2, H-9a), 3.70 (1 H, ddd, $J_{4,3ax}$ 11.4, $J_{4,5}$ 9.8, $J_{4,3eq}$ 4.7, H-4), 3.61 (1 H, dd, $J_{5,6}$ 0.4, $J_{5,4}$ 9.8, H-5), 3.60 (1 H, dd, $J_{9b,9a}$ 10.4, $J_{9b,8}$ 5.7, H-9b), 3.54 (1 H, dd, $J_{6,5}$ 10.4, $J_{6,7}$ 1.9, H-6), 3.52 (1 H, dd, $J_{7,8}$ 8.8, $J_{7,6}$ 1.9, H-7), 2.63 (1 H, dd, $J_{3eq,3ax}$ 12.6, $J_{3eq,4}$ 4.7, H-3_{eq}), 2.48-2.38 (2 H, m, CH_2), 1.99 (3 H, s, AcCH_3), 1.45 (1 H, dd, $J_{3ax,3eq}$ 12.6, $J_{3ax,4}$ 11.4, H-3_{ax}); δ_C (125 MHz, [D_4]MeOH) 175.47 (Ac*C*=O), 134.62 (*C*H=CH_2), 129.29 (arom. C), 128.80 (arom. C), 128.51 (arom. C), 117.87 (CH=*C*H_2), 75.31 (C-6), 74.30 (Ph*C*H_2), 72.75 (C-9), 71.98 (C-8), 70.34 (C-7), 69.78 (C-4), 54.47 (C-5), 45.95 (*C*H_2), 42.16 (C-3), 22.54 (AcCH_3); HRMS (FAB): *m*/*z* calcd. for [M+H]$^+$: 424.197142; found: 424.196808.

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(*E*)-Methyl-5-acetamido-2,6-anhydro-3,5-dideoxy-8,9-*O*-isopropyliden-2-*C*-(3-phenylprop-2-enyl)-D-*erythro*-L-*manno*-nononate (27). Compound **7a** (80 mg, 0.19 mmol) was dissolved in dry acetone (27 ml) under argon atmosphere. After addition of 2,2-dimethoxypropane (240 µl, 1.95 mmol) and ion exchanger (DOWEX 50 × 8, H^+, 80 mg) the solution was stirred overnight at room temperature. When the consumption of starting material was complete (TLC) the yellow solution was filtered, the solvent removed *in vacuo* and the residue purified by column chromatography (dichloromethane/methanol, containing 0.5% triethyl amine) to give **27** (70 mg, 80%); mp 178 °C; $[\alpha]_D^{25}$ +47 (*c* 0.15 in CH_2Cl_2); δ_H (400 MHz, $CDCl_3$) 7.34–7.26 (4 H, m, arom. H), 7.24–7.19 (1 H, m, arom. H), 6.41 (1 H, d, *J* 15.9, CH=C*H*–Ph), 6.08 (1 H, ddd, *J* 15.9 and 2 × 7.5, -C*H*=CH–Ph), 5.62 (1 H, d, $J_{NH,5}$ 7.7, N*H*), 4.32 (1 H, ddd, $J_{8,7}$ 6.4, $J_{8,9a}$ 6.3, $J_{8,9b}$ 6.3, H-8), 4.14 (1 H, dd, $J_{9a,9b}$ 8.6, $J_{9a,8}$ 6.3, H-9a), 4.07 (1 H, dd, $J_{9b,9a}$ 8.6, $J_{9b,8}$ 6.3, H-9b), 3.80 (1 H, ddd, $J_{5,6}$ 10.1, $J_{5,4}$ 10.0, $J_{5,NH}$ 7.7, H-5), 3.75-3.66 (1 H, m, H-4), 3.71 (3 H, s, OCH_3), 3.58 (1 H, dd, $J_{7,8}$ 6.4, $J_{7,6}$ 1.0, H-7), 3.37 (1 H, dd, $J_{6,5}$ 10.1, $J_{6,7}$ 1.0, H-6), 2.73–2.60 (3 H, m, CH_2, H-3$_{eq}$), 2.05 (3 H, s, AcCH_3), 1.62 (1 H, dd, $J_{3ax,3eq}$ 13.0, $J_{3ax,4}$ 11.0, H-3$_{ax}$), 1.40 (3 H, s, C(CH_3)$_2$), 1.37 (3 H, s, C(CH_3)$_2$); δ_C (100 MHz, $CDCl_3$) 173.10, 172.85 (Ac*C*=O), 137.10 (arom. C), 134.38 (CH=*C*H–Ph), 128.71, 127.69, 126.41 (arom. C), 122.79 (-*C*H=CH–Ph), 108.71 (*C*(CH$_3$)$_2$), 81.11 (C-2), 75.74 (C-8), 75.55 (C-6), 70.16 (C-7), 79.11 (C-4), 66.89 (C-9), 53.86 (C-5), 52.29 (OCH$_3$), 43.64 (CH_2), 40.50 (C-3), 26.98, 25.70 (C(CH_3)$_2$), 23.40 (AcCH_3); HRMS (FAB): *m*/*z* calcd. for $[M+H]^+$: 464.228442; found: 464.228058.

(*E*)-Methyl-5-acetamido-2,6-anhydro-3,5-dideoxy-8,9-*O*-isopropyliden-2-*C*-(3-phenylprop-2-enyl)-D-*glycero*-D-*galacto*-non-4-ulosonate (28). Compound **27** (70 mg, 0.15 mmol) was dissolved in dry dichloromethane (4 mL). After addition of powdered mol sieve (4 Å, 280 mg) and pyridinium chlorochromate (130 mg, 603 µmol) the solution was stirred at room temperature until TLC showed complete conversion. The solution was diluted with diethyl ether, filtrated and concentrated *in vacuo*. The residue was purified by column chromatography (dichloromethane/methanol) to give **28** (45 mg, 65%) as a colourless oil; $[\alpha]_D^{25}$ +21 (*c* 0.2 in CH_2Cl_2); δ_H (500 MHz, $CDCl_3$) 7.38–7.29 (5 H, m, arom. H), 6.47 (1 H, d, *J* 15.7, CH=C*H*–Ph), 6.15 (1 H, d, *J* 15.7, *J* 7.5, -C*H*=CH–Ph), 4.67 (1 H, dd, $J_{5,6}$ 10.7, $J_{5,NH}$ 6.8, H-5), 4.09 (1 H, ddd, $J_{8,7}$ 9.0, $J_{8,9b}$ 5.2, $J_{8,9a}$ 3.7, H-8), 3.96 (1 H, dd, $J_{9a,9b}$ 11.2, $J_{9a,8}$ 3.7, H-9a), 3.78 (1 H, dd, $J_{9b,9a}$ 11.2, $J_{9b,8}$ 5.2, H-9b), 3.76 (3 H, s, OCH_3), 3.63 (1 H, dd, $J_{6,5}$ 10.7, $J_{6,7}$ 1.6, H-6), 3.53 (1 H, dd, $J_{7,8}$ 9.0, $J_{7,6}$ 1.6, H-7), 3.22 (1 H, d, $J_{3a,3b}$ 14.0, H-3a), 2.84–2.76 (3 H, m, H-3b, CH_2), 2.09 (3 H, s, AcCH_3), 1.37 (3 H, s, C(CH_3)$_2$), 1.36 (3 H, s, C(CH_3)$_2$); δ_C (125 MHz, $CDCl_3$) 135.63 (CH=*C*H–Ph), 128.68, 127.97, 126.39 (arom. C), 121.21 (*C*H=CH–Ph), 111.89 (*C*(CH$_3$)$_2$), 83.47 (C-2), 78.54 (C-6), 70.62 (C-8), 70.10 (C-7), 64.56 (C-9), 56.95 (C-5), 53.36 (OCH$_3$), 46.63 (C-3), 44.04 (*C*H$_2$), 27.74, 26.42 (C(CH_3)$_2$), 23.15 (AcCH_3); HRMS (FAB): *m*/*z* calcd. for $[M+H]^+$: 462.212792; found: 462.211456.

Methyl-5-acetamido-2,6-anhydro-3,4,5-trideoxy-4-ethoxy-carbonylmethyliden-8,9-*O*-iso-propyliden-2-*C*-((*E*)-3-phenyl-prop-2-enyl)-D-*glycero*-D-*galacto*-nononate (29). Compound **28** (36 mg, 78 µmol) was dissolved in dry tetrahydrofuran and ethoxycarbonylmethylene triphenylphosphorane (54 mg, 0.16 mmol) was added under argon atmosphere. The solution was stirred for 24 h at room temperature. When TLC showed complete conversion the solution was filtered, concentrated *in vacuo* and the residue was purified by column chromatography (dichloromethane/methanol) to give **29** (19 mg, 45%) as a white solid; mp 122 °C; $[\alpha]_D^{25}$ −38 (*c* 0.15 in MeOH); δ_H (500 MHz, [D$_4$]MeOH) 7.37–7.35 (2 H, m, arom. H), 7.30–7.26 (2 H, m, arom. H), 7.22–7.18 (1 H, m, arom. H), 6.47 (1 H, d, *J* 15.9, CH=C*H*–Ph), 6.23 (1 H, ddd, *J* 15.9 and 2 × 7.5, -C*H*=CH–Ph), 5.76 (1 H, dd, *J* 1.3 and 1.2, =*H*C–CO$_2$Et), 4.72 (1 H, dd, $J_{5,6}$ 10.4, *J* 1.2, H-5), 4.46 (1 H, d, $J_{3a,3b}$ 13.9, H-3a), 4.26 (1 H, ddd, $J_{8,9b}$ 7.0, $J_{8,9a}$ 6.5, $J_{8,7}$ 5.2, H-8), 4.19–4.13 (2 H, m, OCH_2CH$_3$), 4.09 (1 H, dd, $J_{9a,9b}$ 8.4, $J_{9a,8}$ 6.5, H-9a), 4.04 (1 H, dd, $J_{9b,9a}$ 8.4, $J_{9b,8}$ 7.0, H-9b), 3.82 (1 H, dd, $J_{6,5}$ 10.4, $J_{6,7}$ 1.2, H-6), 3.76 (1 H, dd, $J_{7,8}$ 5.2, $J_{7,6}$ 1.2, H-7), 3.62 (3 H, s, OCH_3), 2.71 (2 H, d, *J* 7.5, -CH_2CH=), 2.30 (1 H, dd, $J_{3b,3a}$ 13.9, *J* 1.3, H-3b), 2.03 (3 H, s, AcCH_3), 1.39 (3 H, s, C(CH_3)$_2$), 1.33 (3 H, s, C(CH_3)$_2$), 1.26 (3 H, t, *J* 7.1, OCH$_2$CH_3); δ_C (125 MHz, [D$_4$]MeOH) 154.62 (C-4), 135.19 (CH=*C*H–Ph), 129.55, 128.44, 127.27 (arom. *C*), 124.43 (-*C*H=CH–Ph), 114.90 (=H*C*–CO$_2$Et), 84.66 (C-2), 78.25 (C-6), 78.23 (C-8), 70.74 (C-7), 67.17 (C-9), 61.21 (O*C*H$_2$CH$_3$), 52.35 (O*C*H$_3$), 51.81 (C-5), 44.45 (-*C*H$_2$CH=), 36.92 (C-3), 26.92, 25.78 (C(*C*H$_3$)$_2$), 22.73 (Ac*C*H$_3$), 14.56 (OCH$_2$*C*H$_3$); HRMS (FAB): *m*/*z* calcd. for $[M+H]^+$: 532.254657; found: 532.253052.

Methyl-5-acetamido-2,6-anhydro-3,4,5-trideoxy-4-ethoxy-carbonylmethyl-8,9-*O*-isopropyliden-2-*C*-(3-phenylpropyl)-D-*erythro*-L-*manno*-nononate (30). Compound **29** (60 mg, 0.11 mmol), was hydrogenated according to **GP5** to give **30** (60 mg, 100%) as a white solid; mp 115 °C; $[\alpha]_D^{25}$ −73 (*c* 0.1 in MeOH); δ_H (500 MHz, [D$_4$]MeOH) 7.27–7.22 (2 H, m, arom. H), 7.18–7.13 (3 H, m, arom. H), 4.22 (1 H, ddd, $J_{8,7}$ 7.0, $J_{8,9b}$ 6.9, $J_{8,9a}$ 6.5, H-8), 4.15–4.08 (2 H, m, OCH_2CH$_3$), 4.04 (1 H, dd, $J_{9a,9b}$ 8.4, $J_{9a,8}$ 6.5, H-9a), 3.97 (1 H, dd, $J_{9b,9a}$ 8.4, $J_{9b,8}$ 6.9, H-9b), 3.73–3.67 (1 H, m, H-5), 3.71 (3 H, s, OCH_3), 3.61 (1 H, dd, $J_{7,8}$ 7.0, $J_{7,6}$ 1.5, H-7), 3.54 (1 H, dd, $J_{6,5}$ 9.9, $J_{6,7}$ 1.5, H-6), 2.65–2.51 (2 H, m, CH_2c), 2.49 (1 H, dd, *J* 15.4 and 4.3, CH_2CO$_2$Et), 2.33 (1 H, dd, $J_{3eq,3ax}$ 13.4, $J_{3eq,4}$ 3.6, H-3$_{eq}$), 2.11 (1 H, dd, *J* 15.4 and 8.7, CH_2CO$_2$Et), 2.03–1.96 (1 H, m, H-4), 1.94 (3 H, s, AcCH_3), 1.73–1.68 (3 H, m, CH_2a, CH_2b), 1.54–1.46 (1 H, m, CH_2b), 1.41–1.33 (1 H, m, H-3$_{ax}$), 1.37 (3 H, s, C(CH_3)$_2$), 1.33 (3 H, s, C(CH_3)$_2$), 1.25 (3 H, t, *J* 7.1, OCH$_2$CH_3); δ_C (125 MHz, [D$_4$]MeOH) 129.41 (arom. C), 129.35 (arom. C), 126.87 (arom. C), 109.54 (*C*(CH$_3$)$_2$), 81.61 (C-2), 78.05 (C-8), 77.04 (C-6), 70.55 (C-7), 67.09 (C-9), 61.64 (O*C*H$_2$CH$_3$), 52.40 (C-5), 50.52 (O*C*H$_3$), 40.67 (*C*H$_2$a), 38.88 (C-3), 38.77 (*C*H$_2$CO$_2$Et), 36.69 (*C*H$_2$c), 36.28 (C-4), 26.96 (C(*C*H$_3$)$_2$), 26.62 (*C*H$_2$b), 25.81 (C(*C*H$_3$)$_2$), 22.55 (Ac*C*H$_3$), 14.54 (OCH$_2$*C*H$_3$); HRMS (FAB): *m*/*z* calcd. for $[M+H]^+$: 536.285957; found: 536.286560.

Methyl-5-acetamido-2,6-anhydro-3,4,5-trideoxy-4-ethoxy-carbonylmethyl-2-*C*-(3-phenylpropyl)-D-*erythro*-L-*manno*-nononate (31). Compound **30** (58 mg, 0.11 mmol) was suspended in water and a catalytic amount of ion exchanger (DOWEX 50 × 8, H^+) was added. The suspension was stirred overnight at 60 °C and became clear during the course of the reaction. After TLC showed complete consumption of the starting material the solution was filtered and freeze dried to give **31** (40 mg, 75%) as a white solid; mp 62 °C; $[\alpha]_D^{25}$ −42 (*c* 0.1 in MeOH); δ_H (500 MHz, [D$_4$]MeOH) 7.26–7.22 (2 H, m, arom. H), 7.16–7.13 (3 H, m, arom. H), 4.18–4.08 (2 H, m, OCH_2CH$_3$), 3.86–3.80 (2 H, m, H-8, H-9a), 3.79 (3 H, s, OCH_3), 3.69 (1 H, dd, $J_{5,4}$ 10.5, $J_{5,6}$ 10.2, H-5), 3.65–3.60 (1 H, m, H-9b), 3.58 (1 H, dd, $J_{6,5}$ 10.2, $J_{6,7}$ 1.5, H-6),

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3.49 (1 H, dd, $J_{7,8}$ 8.6, $J_{7,6}$ 1.5, H-7), 2.62–2.51 (3 H, m, CH_2c, CH_2CO$_2$H), 2.43 (1 H, dd, $J_{3eq,3ax}$ 13.3, $J_{3eq,4}$ 3.7, H-3$_{eq}$), 2.19–2.12 (1 H, m, CH_2CO$_2$H), 2.04–1.98 (1 H, m, H-4), 1.96 (3 H, s, AcCH_3), 1.75–1.67 (3 H, m, CH_2a, CH_2b), 1.50–1.41 (1 H, m, CH_2b), 1.45 (1 H, dd, $J_{3ax,3eq}$ 13.3, $J_{3ax,4}$ 12.7, H-3$_{ax}$), 1.26 (3 H, t, J 7.1, OCH$_2$CH_3); δ_C (100 MHz, [D$_4$]MeOH) 179.27 (C-1), 175.86 (CH$_2$CO$_2$Et), 129.42 (arom. C), 129.36 (arom. C), 126.88 (arom. C), 81.54 (C-2), 76.59 (C-6), 73.05 (C-8), 70.26 (C-7), 64.57 (C-9), 61.67 (OCH$_2$CH$_3$), 52.97 (OCH$_3$), 50.51 (C-5), 43.66 (CH$_2$a), 39.19 (C-3), 38.51 (CH$_2$CO$_2$Et), 36.63 (CH$_2$c), 36.41 (C-4), 26.49 (CH$_2$b), 22.51 (AcCH$_3$), 14.55 (OCH$_2$CH$_3$); MS (MALDI-TOF): m/z 496.5 ([M+H]$^+$), 518.5 ([M+Na]$^+$), 534.4 ([M+K]$^+$).

5-Acetamido-2,6-anhydro-3,4,5-trideoxy-4-carboxymethyl-2-*C*-(3-phenylpropyl)-D-*erythro*-L-*manno*-nononic acid (32). Compound **31** (40 mg, 81 μmol) was saponified according to **GP4** to give **32** (18 mg, 49%) as a white solid; mp 183–185 °C; $[\alpha]_D^{25}$ −32.2 (c 0.45 in MeOH); δ_H (400 MHz, [D$_4$]MeOH) 7.23–7.07 (5 H, m, arom. H), 3.86 (1 H, ddd, $J_{8,7}$ 8.9, $J_{8,9b}$ 5.6, $J_{8,9a}$ 2.6, H-8), 3.80 (1 H, dd, $J_{9a,9b}$ 11.3, $J_{9a,8}$ 2.6, H-9a), 3.62–3.56 (2 H, m, H-6, H-9b), 3.46 (1 H, dd, $J_{7,8}$ 8.9, $J_{7,6}$ 2.1, H-7), 3.44 (1 H, dd, $J_{5,4}$ 10.4, $J_{5,6}$ 10.4, H-5), 2.58–2.52 (2 H, m, CH_2c), 2.52 (1 H, dd, $J_{3eq,3ax}$ 13.2, $J_{3eq,4}$ 3.5, H-3$_{eq}$), 2.34 (1 H, dd, J 14.5 and 5.4 Hz, CH_2COOH), 2.16–2.09 (1 H, m, H-4), 2.04 (1 H, dd, J 14.5 and 6.6, CH_2COOH), 1.96 (3 H, s, AcCH_3), 1.88–1.59 (4 H, m, CH_2a, CH_2b), 1.26 (1 H, dd, $J_{3ax,3eq}$ 13.2, $J_{3ax,4}$ 12.3, H-3$_{ax}$). δ_C (100 MHz, [D$_4$]MeOH) 179.86 (C-1), 175.26 (CH$_2$COOH), 129.47 (arom. C), 129.16 (arom. C), 126.51 (arom. C), 82.78 (C-2), 76.89 (C-6), 72.95 (C-8), 70.79 (C-7), 64.67 (C-9), 52.98 (C-5), 42.52 (CH$_2$a), 41.92 (CH$_2$COOH), 41.12 (C-3), 37.40 (CH$_2$c), 37.03 (C-4), 27.24 (CH$_2$b), 22.49 (AcCH$_3$); HRMS (FAB): m/z calcd. for [M+H]$^+$: 454.207707; found: 454.209564.

Affinity measurements by SPR

Immobilisation of the enzyme on the sensor chip. 120 μL of a TcTS solution in phosphate buffer (100 mM, pH 7.5, 0.17 mg mL^{-1}) was diluted with 880 μL of acetate buffer (10 mM, pH 6.06). A commercial CM5 sensor chip (BIACORE) was activated in the BIACORE T100 instrument by flushing with EDC/NHS solution for 10 min. and the enzyme was immobilised on the chip by automatically injecting the prepared solution for 10 min. This step was repeated once (for 20 min) to yield an overall response of approximately 12000 RU (response units). The flow rate in all steps was 10 μL/min. The chip can be stored in buffer solution at 4 °C for months without losing activity.

Surface plasmon resonance measurements. The ligand was dissolved in running buffer (Tris-HCl, 100 mM, pH 7.46) to yield a 5 mM solution. Different concentrations needed for SPR measurements were made by diluting the solution with running buffer.

The affinity measurements were conducted by injecting the concentrations into the BIACORE T100 instrument (contact time 180 s) at a flow rate of 20 μL min^{-1}, followed by flushing with buffer (dissociation time 300 s). A washing step was included between ligand injections. The response was in a range of about 40 to 200 RU for the highest concentrations of the measured ligands.

NMR based inhibition assay

TRIS-HCl buffer (100 mM, pH 7.5) was lyophilised and redissolved in the same volume of D$_2$O twice. The same was done to a solution of TcTS (0.9 mg mL^{-1}) in TRIS-HCl buffer.

Methyl α-allolactoside (35 μmol) and *p*NP-sialoside (25 μmol)[27] were dissolved in 1 mL of TRIS-HCl (D$_2$O) and 80 μL of the TcTS solution (D$_2$O) were added. The mixture was incubated at 23 °C. Samples (100 μL) were taken after the indicated reaction time (Fig. 6) and added to previously prepared NMR tubes containing a 1 : 1 mixture of D$_2$O/[D$_4$]MeOH. The ratio of free *p*-nitrophenol to *p*NP-sialoside was determined by the ratio of integrated aromatic proton signals in the proton NMR spectra. Since hydrolysis can be neglected in the presence of a suitable acceptor molecule,[3] conversion rates can be measured easily with this method.

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