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Synthetic Strategy towards a Carbocyclic *N*-Acetylneuraminic Acid

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In the study of glycosidases, a class of activity-based probes (ABPs), that are carbocyclic mimics of natural carbohydrates and can covalently bind the enzyme, have proven to be useful tools. This type of ABP has however not yet been reported for sialidases, glycosidases involved in various important biological processes in both health and disease, which hydrolyse terminal sialic acids. Here we present our study towards the synthesis of a carbocyclic sialic acid suitable for conversion into ABPs. We developed a route starting from a chiral furanone that includes a key early stage nitrone [3+2] cycloaddition to install most of the chiral centres present in *N*-acetylneuraminic acid. The final stereocentre is installed via a Barbier alkylation, after which a ring closing metathesis forms the pivotal carbocyclic intermediate. Due to challenges in the final stretch, we were not able to convert this intermediate into an *N*-acetylneuraminic acid ABP. However, the work presented here still represents a versatile route to potential future carbocyclic sialic acid derivatives.

Introduction

Sialic acids decorate the termini of various oligosaccharides and are vital for many processes, both inter- and intracellular.[1] For example, sialylated extracellular oligosaccharides serve as a recognition element for Siglecs on immune cells, regulating their activity.[2] In mammals, *N*-acetylneuraminic acid (Neu5Ac) is the predominant sialic acid, the level of expression of which is vital, as both over-, and under expression of sialic acid on glycans can cause various disorders, such as HIBM, sialuria, Salla disease, ISSD and sialidosis.[3]

Sialidases are the glycosyl hydrolase enzymes, also called glycosidases, responsible for cleaving sialic acids from the termini of glycans and are found in both vertebrates and various microbes. The activity of sialidases directly impacts the level of sialic acid on cells,[4] allows the scavenging of sialic acid from other cells for use as an energy source[5] or is part of infection mechanisms of both viral and bacterial pathogens.[6] These various functions in health and disease, and the many different types of sialidases make this class of glycosidases an interesting target for study. The development of molecular tools that can perturb sialidase activity[7] and assist in their identification and quantification[8] is a key way to gain insight into their functional role(s). Activity-based protein profiling with a chemical probe is a method for studying enzymes that has already been utilised successfully in the past for several glycosidases.[9] This method works by treating a biological sample containing the enzyme of interest with a probe equipped with a warhead (for covalent binding to the protein), a recognition element (for selectivity to the desired protein) and a reporting group (for detection and/or extraction of the desired protein).

Previously reported glycosidase ABPs can be roughly divided in three classes. One class are analogues of the target enzyme substrate with a fluor-group near the anomeric centre,[10] which creates a stabilised covalent intermediate in retaining glycosidases. Another class of probes contain the native glycoside substrate that releases a reactive group, such as a quinone methide, upon enzymatic hydrolysis.[11] These can in principle label both retaining and inverting glycosidases. For both these classes, probes that target sialidases have been reported.[12] These classes have some drawbacks however, non-specific labelling makes off-targets appear as having the studied activity or the, sometimes, large reporter groups cause cell membrane impermeability.

The third class consists of probes based on a carbocyclic mimic of the substrate. These use an electrophilic warhead, either an epoxide, aziridine or cyclic sulphate to trap the catalytic nucleophile and covalently label the enzyme.[13] In this class, promising ABPs for various glycosyl hydrolases have been published in the past decade which were highly selective and effective in much lower concentrations than other classes of ABPs.[14] However, a carbocyclic sialidase ABP has not been developed yet.

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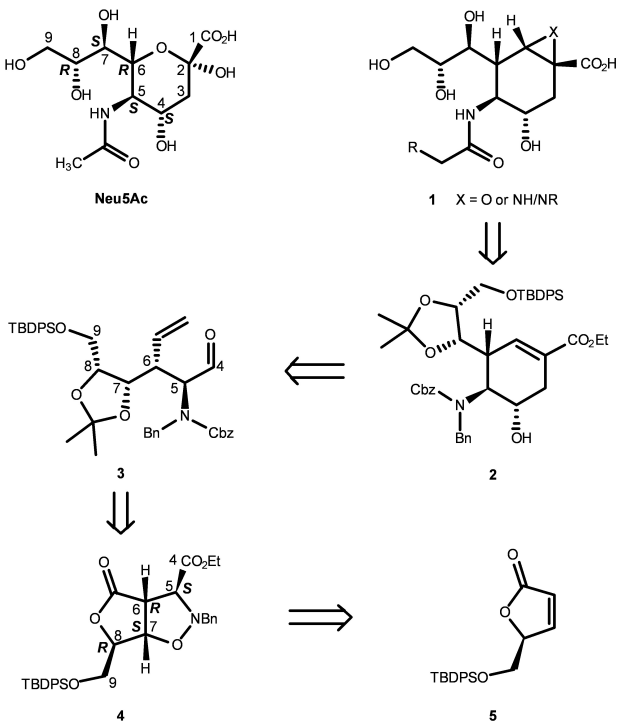


Scheme 1. Structure of Neu5Ac and retrosynthesis towards a carbocyclic sialic acid mimic.

A carbocyclic version of Neu5Ac has been made before by Vasella and co-workers via an approach starting from *N*-acetyl-D-mannosamine.[15] There are however two obstacles to using this route for development of an ABP. First, since the route starts with an *N*-acetyl already in place; it can no longer be functionalised with a reporting group on this position. The second obstacle is that in the step that forms the endocyclic olefin, via elimination of a phenylselenide. the selectivity laid mostly towards the undesired isomer with the alkene between the pseudo C1 and C2 of Neu5Ac.

In this study we therefore set out to develop a new synthetic route to a carbocyclic version of Neu5Ac that would be suitable for further functionalisation by installation of a warhead and a click handle to produce ABPs for the study of sialidases.

To obtain a carbocyclic mimic of Neu5Ac that could be functionalised in these versatile ways, we envisioned key intermediate **2** as shown in Scheme 1. This intermediate contains an alkene in the carbocycle for installing an electrophilic trap, such as an epoxide, aziridine or cyclic sulphate warhead. Another feature is the protected amine on the C5 position which can be selectively deprotected to install either the natural acetate group or acylated with a reporter group such as a click handle, biotin moiety or a fluorophore. We envisioned a ring closing metathesis of a linear diene precursor as a key late-stage step to form the carbocycle. The diene itself would be formed via a Barbier reaction on aldehyde **3**.

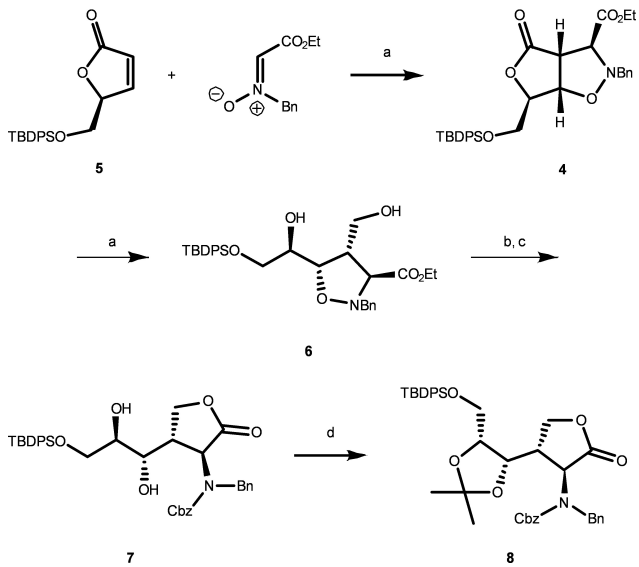
This crucial aldehyde intermediate (**3**), with the correct Neu5Ac stereochemistry (see Scheme 1), is obtained after a few transformations from isoxazolidine-lactone bicycle **4**, which can, in turn be obtained via a 3+2 cycloaddition of a nitrone to a readily accessible D-mannitol derived furanone **5**.

Results and Discussion

We previously reported the synthesis of various bicyclic isoxazolidine lactones that could be prepared in a straightforward manner via a [3+2] cycloaddition of furanones and nitrones.[16] These compounds were utilised to synthesise various glycomimetics starting from the D-mannitol derived (5*S*)-5-(hydroxymethyl)-furanones (see **5** in Scheme 2). During this study we realised that isoxazolidine-lactone bicycle **4** contained the correct sequence of stereocentres for C5 to C9 of Neu5Ac.

We initially considered using a derivative of **4**, made with a nitrone functionalised with a dimethyl acetal masked aldehyde, as our starting point towards a carbocyclic Neu5Ac. However, when we discovered that a selective reductive opening of the lactone in **4**, resulting in compound **6**, could also act as a convenient starting point for a synthetic route towards a carbocyclic Neu5Ac (**2**), we chose this as our starting point.

The development of this route started by opening the isoxazolidine ring in **6** using Raney-nickel. To our surprise this exclusively yielded the lactone, which was probably possible due to the primary hydroxyl gaining the freedom of movement necessary to perform spontaneous intramolecular trans-esterification on the ethyl ester. Subsequent carbamate-protection of the amine as a benzyloxy (Cbz) carbamate gave lactone **7**. Protection of the diol in **7** using 2,2-dimethoxypropane as an isopropylidene acetal afforded compound **8** in 84% yield over 3 steps.

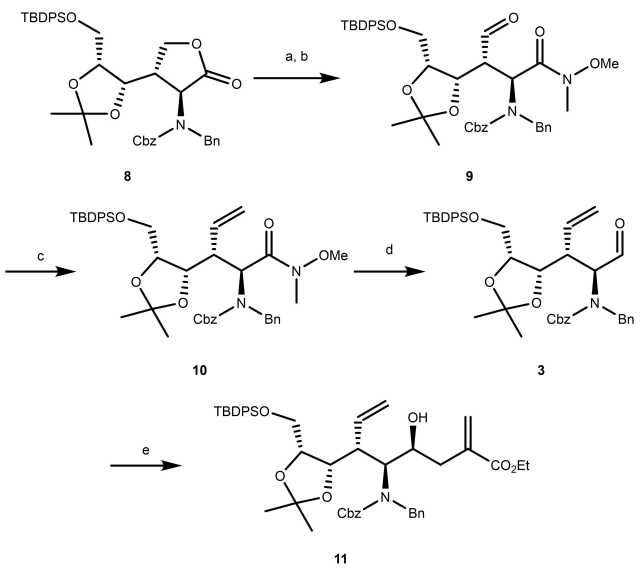


Scheme 2. Synthesis of the C4 to C9 chain of the carbocyclic sialic acid target. a) 4 steps, 50% overall yield, previous work[16] b) Raney-Ni, H_2, THF, RT, 4 h. c) Cbz-Cl, $NaHCO_3$, THF, RT, 18 h. d) 2,2-dimethoxypropane, CSA, DCM, RT, 1.5 h. 84% over 3 steps.

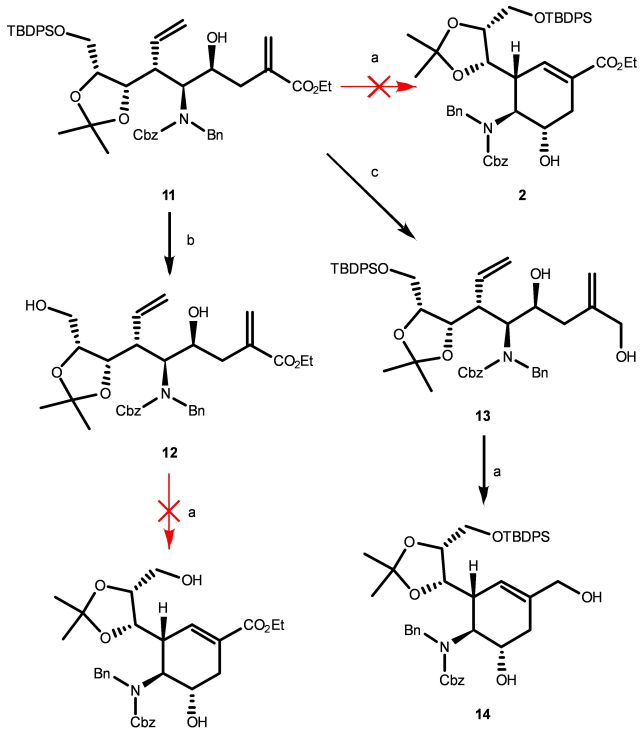
The lactone was opened using trimethyl aluminium and methoxy methylamine to create a Weinreb amide. While the conversion to the newly formed primary hydroxyl was complete and the compound was stable on analytical TLC, we were surprised to discover quantitative cyclisation back to the lactone during chromatographic purification. To prevent this; the primary alcohol was oxidised directly after work-up of the Weinreb amide using Dess-Martin periodinane. In this manner aldehyde **9** was obtained in 67% yield over 2 steps and reversion back to **8** was no longer possible.

The next step was to convert the aldehyde into an olefin. However, we wanted to avoid possible epimerisation at the stereocentre adjacent to the aldehyde under the basic conditions of a traditional Wittig reaction. Therefore, we used modified Wittig conditions that avoid the use of a strong base, using a rhodium catalyst, as reported by Lebel et. al..[17] With these conditions, we observed successful conversion of aldehyde **9** to compound **10**, containing the first alkene needed for ring closing metathesis, in 79% yield as shown in Scheme 3. Reduction of the Weinreb amide using $LiAlH_4$ gave aldehyde **3**, which was used in crude form for the subsequent Barbier reaction.

Treatment of the aldehyde with ethyl-2-(bromomethyl)-acrylate in the presence of Indium powder gave diene **11** as a 7/3 mixture, which could be partially separated, in 70% yield over 2 steps. Determining the stereochemistry of the two diastereomers by NMR was not possible due to signal broadening, probably caused by the carbamate protecting group. However, the Barbier reaction has been performed numerous times on similar substrates[18] and always favoured the formation of the product with a *syn*-relationship between the chiral centre, originally at the aldehyde alpha carbon, and the newly created stereocentre, which in this system is the desired Neu5Ac configured stereoisomer.



Scheme 3. Synthesis of the diene. a) $AlMe_3$, HN(OMe)Me, DCM, −80 °C, 5 h. b) Dess-Martin periodinane, $NaHCO_3$, DCM, 0 °C, 1 h, 67% over 2 steps. c) $RhCl(PPh_3)_3$, PPh_3, $TMSCH_2N_2$, *i*PrOH, THF, RT, 3 h, 78% yield. d) $LiAlH_4$, THF, −80–0 °C, 1.5 h. e) ethyl (2-bromomehtyl) acrylate, Indium, $EtOH/H_2O$, sonication, 35–40 °C, 18 h, 70% yield over 2 steps.



Scheme 4. Ring closing issues and solutions. a) Grubbs catalyst (2nd gen. or C571), toluene, 80 °C, 4 h (45% yield for **15**). b) HF/pyridine, DCM, 0 °C, 3 h, 30% yield. c) DiBAl–H, toluene, −78 °C, 3 h, 69% yield.

We next attempted to perform ring closing metathesis (RCM) on the system, but treatment of **11** with 2nd generation Grubbs catalyst, as shown in Scheme 4, unfortunately did not result in any reaction. Running the reaction in $CDCl_3$ and monitoring by ^{1}H-NMR indicated no formation of the Ru=CH_2 resting state of the catalyst, normally formed after initiation of the metathesis cycle. Addition of diallyl ether did produce Ru=CH_2, but still did not lead to cyclisation of **11** into **2**. We hypothesised that the cause of the issue was either steric hinderance of the bulky protecting groups or an electronic effect from the ester moiety conjugated with one of the olefins. We attempted to eliminate the factor of steric hinderance by removing the TBDPS using HF/pyridine giving compound **12** in 30% yield.

However, this still did not undergo the RCM. Switching to a different catalyst, Hoveyda-Grubbs C571, which was developed to catalyse RCM in sterically hindered systems, also did not result in any conversion. To explore the electronic effect on metathesis, we next reduced the ester group in **11** using DiBAl–H, to obtain the primary alcohol **13** in 69% yield. Fortunately, compound **13** did convert to the olefinic carbocycle **14** in 45% yield using the specialised Hoveyda-Grubbs C571 catalyst.

Given the result that **13**, with a primary alcohol instead of an ester moiety, did cyclise to the carbocycle we investigated whether elongating the chain of aldehyde **3** could be achieved

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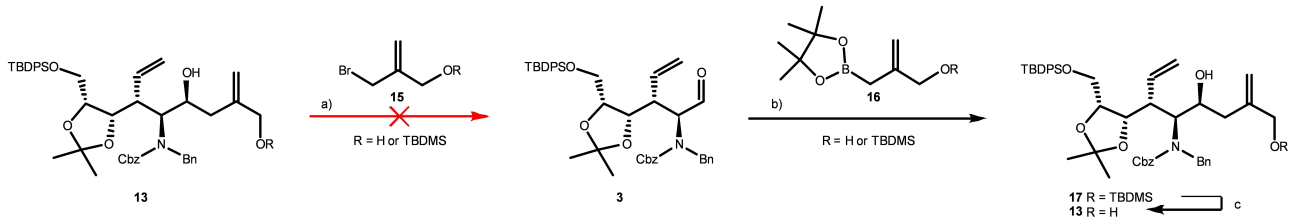


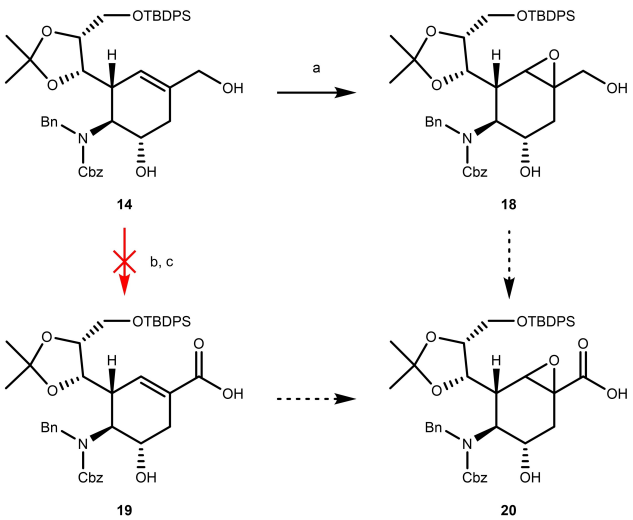
Scheme 5. Alternatives to the Barbier reaction. a) **15**, Indium, EtOH/H_2O, sonication, 35–40 °C, 16 h. b) **16**, toluene, RT, 16 h, 28% yield. c) $CuSO_4$, MeOH, 50 °C, 5 h, 30% yield.

with this required primary alcohol at C9 already in place. The Barbier reaction performed with alkyl bromide **15**, with either a free hydroxyl or silyl ether protected version (Scheme 5), did not result in any formation of the desired product **13**.

We next explored a stereoselective alkylation with pinacol-boronic ester[19] **16**, with either the free alcohol or a silyl ether protected version of the boronic acid reagent. With the TBDMS protected version of **16** only, this reaction afforded the desired product **17** in a lower yield than the Barbier reaction that had produced **11**, but in a favourably higher diastereomeric excess of 9/1. We next investigated the conditions to selectively remove the TBDMS of **17** while preserving the TBDPS. We were able to obtain the desired intermediate **13** in 30% yield, but a major side reaction that occurred with these conditions was partial deprotection of the isopropylidene. This led to multiple partially or fully deprotected products and the low overall yield of **13**.

With carbocycle **14** in hand, we wanted to evaluate whether it would be possible to oxidise the primary alcohol back to the carboxylic acid as shown in Scheme 6 and functionalise the alkene with an epoxide warhead.

We investigated oxidation of **14** to carboxylic acid **19** that would be followed by general deprotection and epoxidation to obtain the final product (**1**). However, when attempting to oxidise the primary alcohol in **14** using TEMPO/BAIB, the oxidation halted at the aldehyde stage and no trace of carboxylic acid **19** was detected. A subsequent attempt to oxidise **14** via the Pinnick oxidation unfortunately also did not result in the formation of carboxylic acid **19**. Reversing the sequence to first perform the epoxidation and then the oxidation was investigated. Treatment of **14** with *m*-CPBA successfully produced compound **18** in 63% yield. However, we were concerned that the epoxide might not be stable during deprotection of the isopropylidene acetal and no longer had sufficient material to investigate further conditions.



Scheme 6. Functionalising the ring. a) *m*CPBA, DCM, 0 °C, 48 h, 63% yield. b) TEMPO, BAIB, DCM/H_2O, RT, 3 h. c) $NaClO_2$, NaH_2PO_4, resorcinol, *t*BuOH, RT.

Conclusions

Starting out from the earlier reported bicyclic isoxazolidine-lactone **4** containing many of the stereocentres required to mimic Neu5Ac, we successfully developed a route to obtain carbocycle **14**. While further functionalisation to obtain a sialidase ABP has yet to be successful, our route provides a reliable method of obtaining a highly complex versatile building block for synthesising carbocyclic Neu5Ac mimics. The route starting from chiral furanone **5** includes a crucial early stage nitrone [3 + 2] cycloaddition, the formation of a Weinreb amide from a lactone with an accompanying oxidation and rhodium catalysed Wittig sequence on the thereby liberated hydroxyl. After installation of a first alkene, a Barbier alkylation installs the second and a ring closing metathesis enables the synthesis of the key carbocyclic intermediate with all the correct stereocentres.

In performing this route, the largest challenges were found in the ring closing metathesis and the reintroduction of the carboxylic acid together with a suitable warhead. Further optimisation and exploration of these reactions is possible, so this route could still become a versatile method to obtain the desired activity-based probe class (**2**). The steps that would still need to be done are the oxidation of the primary alcohol in the presence of the epoxide, or an alternative warhead in the form of an aziridine or cyclic sulphate. Alternatively, it might be possible to identify conditions that enable oxidation of the allylic alcohol of **14** to a carboxylic acid, followed by deprotection and warhead introduction. The final deprotection of the protected amine with Pd/C catalysed hydrogenolysis and subsequent acetylation of the amine with a reporter group will probably prove possible.

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Experimental Section

General procedures

All moisture sensitive reactions were carried out under an argon atmosphere in glassware that was dried by heating (~100 °C) under vacuum. Anhydrous solvents were obtained from a solvent purification system or stored on activated mol. sieves 4 Å. All commercially available reagents were used as supplied. Analytical TLC was performed using prepared plates of silica gel (60 F254, either aluminium or glass backed). Visualisation of TLC results was done by short wave UV light and either a basic $KMnO_4$ or an ammonium molybdate solution. Purification by column chromatography was performed with 70–230 mesh silica gel. The NMR spectra were recorded on a 600 MHz Agilent or a 400 MHz Bruker spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to traces of the non-deuterated solvent in the corresponding deuterated solvent. High-resolution mass analyses were recorded on an Orbitrap high-resolution mass spectrometer.

Benzyl benzyl((3S,4S)-4-((1S,2R)-3-((tert-butyldiphenylsilyl)oxy)-1,2-dihydroxypropyl)-2-oxotetrahydrofuran-3-yl)carbamate (7)

To a round-bottom flask containing Raney-Ni (12.0 g), washed beforehand with anhydrous THF (×2), was added a solution of diol **6** (6.12 g, 9.39 mmol) in anhydrous THF (90 mL). The resulting grey slurry was vigorously stirred under H_2 for 4 h. Crude ^{1}H-NMR indicated complete conversion (TLC-analysis is *not* recommended). The H_2 atmosphere was replaced with an argon atmosphere. H_2O (48 mL), $NaHCO_3$ (2.9 g, 34.5 mmol 3.67 equiv.) and CbzCl (4.95 mL, 34.6 mmol, 3.69 equiv.) were sequentially added and the mixture was stirred for 18 h. Sat. aq. $NaHCO_3$ (150 mL) was added, and the mixture was filtered over celite, which was washed with additional THF. The resulting filtrate was extracted with EtOAc (3×350 mL), the combined organic layers were washed with brine (200 mL), dried with $MgSO_4$ and conc. *in vacuo*, affording the crude product (8.49 g) as a dark pink oil, which was used without further purification. Optionally, the crude could be purified by flash column chromatography (9/1→7/3 PE/EtOAc) to yield compound **7** as a white amorphous solid. R_F = 0.23 (7/3; PE/EtOAc). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.62 (d, *J* = 7.4 Hz, 4H), 7.53–7.11 (m, 18H), 5.19 (s, 2H), 4.79 (d, *J* = 15.4 Hz, 1H), 4.71 (d, J = 5.6 Hz, 1H), 4.48 (d, J = 15.9 Hz, 1H), 4.26 (t, J = 8.6 Hz, 1H), 4.22–4.07 (m, 1H), 4.03 (d, J = 9.3 Hz, 1H), 3.67–3.50 (m, 1H), 3.30 (d, J = 41.3 Hz, 2H), 3.06 (s, 1H), 2.72 (s, 1H), 1.06 (s, 9H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 173.24, 156.82, 137.7, 135.5, 132.6, 130.2, 128.7, 128.6, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 72.0, 68.3, 67.3, 65.0, 57.3, 56.9, 50.2, 41.9, 41.7, 29.7, 26.9, 19.2, 14.2; LRMS calcd. for $C_{38}H_{43}NO_7Si + Na^+$ [M + Na^+]: 676,27 Found 676.50.

Benzyl benzyl((3S,4S)-4-((4S,5R)-5-(((tertbutyldiphenylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-oxotetrahydrofuran-3-yl) carbamate (8)

Crude compound **7** (8.49 g) was dissolved in DCM (25 mL), followed by the addition of 2,2-dimethoxypropane (5.55 mL, 45.3 mmol, 4.8 equiv.) and camphorsulfonic acid (42.0 mg, 0.181 mmol, 0.019 equiv.). The resulting reaction mixture was stirred for 80 min. and conc. *in vacuo*, affording an orange oil. The crude oil was purified by flash column chromatography (9/1→4/1 PE/EtOAc), affording compound **8** (5.46 g, 84% over 3 steps).* R_F = 0.70 (7/3; PE/EtOAc). ^{1}H-NMR (400 MHz, CDCl3): δ 7.60–7.44 (m, 4H), 7.43–7.17 (m, 16H), 5.21–5.08 (m, 2H), 4.85 (d, J = 15.5 Hz, 1H), 4.32 (d, J = 15.4 Hz, 1H), 4.21–3.77 (m, 4H), 3.56–3.48 (m, 1H), 3.20–3.02 (m, 2H), 1.27–1.03 (m, 6H), 0.96 (d, J = 6.9 Hz, 9H); 13 C-NMR (101 MHz, CDCl3): δ 173.24, 156.82, 137.74, 135.49, 132.60, 130.15, 128.74, 128.6, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 72.0, 68.3, 67.3, 65.0, 57.3, 56.9, 50.2, 41.9, 41.7, 29.7, 26.9, 19.2, 14.2. FT-IR (neat): ν = 2932, 2858, 1782, 1700, 1497, 1456, 1427 cm-1. LRMS calcd. for C41H47NO7Si + Na + [M + Na +]: 716.30.

Found: 716.50.

*NMR characterisation suffered from peak broadening and peak splitting due to the formation of rotamers.

Benzyl benzyl((2S,3R)-3-((4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-1-(methoxy(methyl)amino)-1,4-dioxobutan-2-yl)carbamate (9)

A suspension of (MeO)NHMe·HCl (0.21 g, 2.16 mmol, 3.0 equiv.) in anhydrous DCM (5.0 mL) was cooled to −78 °C, followed by dropwise addition of a solution of $AlMe_3$ (2.0 M in toluene, 1.05 mL, 2.10 mmol, 2.9 equiv.) over 5 min. The mixture was allowed to warm to −35 °C, over 1.5 h, after which it was cooled to −50 °C and a solution of compound **8** in anhydrous DCM (5.0 mL) was added dropwise over 5 min. The resulting reaction mixture was stirred at −50 °C for 20 min, the cooling bath was removed, and the mixture was allowed to warm to room temperature for 3 h. The mixture was poured into sat. aq. Rochelle's salt (25 mL) and the resulting light grey slurry was filtered over celite, which was rinsed with additional DCM. The resulting biphasic system was separated, and the aqueous layer was extracted with DCM (2×20 mL). The combined organic layers were dried with Na_2SO_4 and conc. *in vacuo*, affording the crude primary alcohol intermediate as a colourless oil (0.66 g), R_f = 0.20 (7/3; PE/EtOAc), which was used without further purification. The crude intermediate was dissolved in DCM (15 mL) and cooled to 0 °C, followed by the addition of $NaHCO_3$ (1.00 g, 11.94 mmol, 16.4 equiv.) and Dess-Martin periodinane (0.62 g, 1.46 mmol, 2.0 equiv.). The reaction mixture was stirred at 0 °C for 1 h, diluted with Et_2O (50 mL) and poured in sat. aq. $NaHCO_3$ (50 mL) containing $Na_2S_2O_3$ (6.0 g). This biphasic system was stirred at rt for 5 min., the layers were separated, and the water layer was extracted with Et_2O (3×50 mL). The combined organic layers were washed with brine (50 mL), dried with Na_2SO_4 and conc. *in vacuo.* The resulting yellow oil was purified by flash column chromatography (PE/EtOAc 85/15→4/1) to give compound **9** as a white foam (0.37 g, 0.49 mmol, 67% over 2 steps).* R_F = 0.61 (7/3; PE/EtOAc). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.71 (s, 1H), 7.75–7.59 (m, 4H), 7.49–7.03 (m, 17H), 5.73 (d, *J* = 11.2 Hz, 1H), 5.26 (dd, *J* = 12.4, 2.9 Hz, 1H), 5.21–5.05 (m, 1H), 4.49 (d, *J* = 15.4 Hz, 1H), 4.43–4.26 (m, 1H), 4.20 (d, *J* = 6.5 Hz, 1H), 4.17–3.94 (m, 2H), 3.79–3.63 (m, 4H), 3.42 (s, 2H), 2.76 (d, *J* = 6.9 Hz, 3H), 1.27 (d, *J* = 16.0 Hz, 4H), 1.17 (d, *J* = 20.1 Hz, 4H), 1.09 (d, *J* = 7.8 Hz, 9H). ^{13}C-NMR (101 MHz, $CDCl_3$): δ 200.8, 200.6, 169.4, 169.1, 157.0, 156.4, 137.9, 137.8, 136.4, 136.3, 136.2, 136.2, 136.0, 135.8, 135.7, 132.9, 132.9, 132.8, 130.1, 130.0, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.4, 127.3, 127.1, 108.7, 77.1, 77.0, 74.1, 74.0, 68.1, 68.0, 63.0, 62.8, 61.8, 61.4, 53.9, 49.1, 48.8, 47.2, 47.0, 31.9, 27.3, 27.1, 26.9, 26.8, 24.6, 19.3. FT-IR (neat): ν = 2934, 2860, 1698, 1660, 1428, 1411 cm^{-1}. HRMS calcd for $C_{43}H_{54}N_2O_8Si + Na^+$ M + Na^+]: 777.3547. Found 777.3519. *NMR characterisation suffered from peak broadening and peak splitting due to the formation of rotamers.

Benzyl benzyl((2S,3R)-3-((4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)-methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-1-(methoxy(methyl)amino)-1-oxopent-4-en-2-yl)carbamate (10)

Anhydrous THF (5 mL) was degassed with N_2. *i*PrOH (0.25 mL, 3.27 mmol, 2.5 equiv.), PPh_3 (0.79 g, 3.01 mmol, 2.3 equiv.) and

$Rh(I)(PPh_3)_3Cl$ (75 mg, 0.08 mmol, 6 mol%) were added. **9** (1.00 g, 1.33 mmol) was dissolved in anhydrous THF (15 mL) and degassed with N_2, this solution was added to the red mixture followed by dropwise addition of a solution of TMS-diazomethane (2.0 M in hexanes, 2.0 mL, 4.0 mmol, 3.0 equiv.). The mixture was left to stir at RT for 3 h (precipitation and gas evolution was observed after circa 1 h) and poured into a mixture of DCM (150 mL) and H_2O (150 mL). The biphasic system was separated, and the aqueous layer was extracted with DCM (2×50 mL). The organic layers were combined, dried with Na_2SO_4, filtered and concentrated under reduced pressure. The resulting oil was purified using column chromatography (PE/Et_2O 95:5→9:1→4:1) to give compound **10** as a yellow oil (0.79 g, 1.05 mmol, 79%).* R_F = 0.34 (8/2; PE/EtOAc). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.73–7.53 (m, 5H), 7.50–6.95 (m, 17H), 5.87–5.65 (m, 1H), 5.56 + 5.38 (2xd, J = 11.2 Hz, 1H), 5.25–4.87 (m, 4H), 4.86–4.28 (m, 2H), 4.18–3.80 (m, 2H), 3.76–3.48 (m, 3H), 3.38–2.99 (m, 3H), 2.77 (d, J = 44.7 Hz, 3H), 1.32 (s, 3H), 1.15 (s, 3H), 1.02 (d, J = 10.1 Hz, 9H). ^{13}C-NMR (101 MHz, $CDCl_3$): δ 168.5, 155.4, 137.0, 134.1, 132.0, 128.1, 126.8, 126.6, 126.4, 126.2, 125.4, 118.9, 106.3, 76.4, 72.4, 66.3, 61.3, 60.3, 53.9, 45.4, 39.6, 30.4, 25.4, 23.1, 17.7. LRMS calcd for $C_{44}H_{54}N_2O_7Si + Na^+$ [M + Na^+]: 773.36. Found 773.50. *NMR characterisation suffered from peak broadening and peak splitting due to the formation of rotamers.

Benzyl benzyl((2S,3R)-3-((4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-1-oxopent-4-en-2-yl)carbamate (3)

Weinreb amide **10** (0.79 g, 1.05 mmol) was co-evaporated with anhydrous toluene (×3) and dissolved in anhydrous THF (35 mL). The solution was cooled to −78 °C, followed by the dropwise addition of a solution of $LiAlH_4$ (1.0 M in THF, 2.4 mL, 2.4 mmol, 2.3 equiv.). The mixture was left to stir for 30 min, heated to 0 °C in an ice bath and left to stir for 2 h after which it was cooled back to −78 °C and quenched by addition of sat. aq. NH_4Cl (5.0 mL) followed by the addition of EtOAc (50 mL). The mixture was allowed to warm to RT and stirred for 30 min. Finally, H_2O (50 mL) and additional EtOAc (50 mL) were added, and the biphasic system was separated. The aqueous layer was extracted with EtOAc (2× 50 mL) and the combined organic layers were washed with sat. aq. $NaHCO_3$ (100 mL) and brine (100 mL), dried with Na_2SO_4 and conc. *in vacuo*, affording pure aldehyde **3** (0.74 g, quant.) as a yellow oil.* R_F = 0.53 (85/15 PE/Et_2O). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.55 (s, 1H), 9.27 (s, 1H), 7.77–7.54 (m, 5H), 7.52–7.15 (m, 20H), 5.98–5.75 (m, 1H), 5.23–4.78 (m, 6H), 4.39–4.12 (m, 3H), 3.97–3.84 (m, 1H), 3.83–3.59 (m, 2H), 3.49 (d, J = 4.7 Hz, 2H), 1.42 (s, 3H), 1.30 (s, 3H), 1.07 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.6, 197.2, 156.0, 137.3, 136.2, 135.8, 135.7, 135.3, 133.6, 133.4, 129.8, 129.8, 128.7, 128.7, 128.6, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.8, 127.7, 127.0, 120.5, 108.0, 78.5, 74.8, 68.1, 67.9, 67.5, 62.7, 53.1, 42.4, 42.0, 27.1, 27.0, 26.7, 24.7, 19.3. FT-IR (neat): ν = 2932, 2858, 1731, 1697, 1497, 1456, 1427 cm^{-1}. HRMS calcd for $C_{42}H_{49}NO_6Si + Na^+$ [M + Na^+]: 714.3221. Found 714.3196. *NMR characterisation suffered from peak broadening and peak splitting due to the formation of rotamers.

Ethyl (5S,6R)-5-(benzyl((benzyloxy)carbonyl)amino)-6-((4S,5R)-5-(((-tert-butyldiphenylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-4-hydroxy-2-methyleneoct-7-enoate (11)

Aldehyde **11** (0.74 g, 1.06 mmol), ethyl-2-(bromomethyl)acrylate (0.44 mL, 0.62 g, 3.19 mmol, 3.0 equiv.) and indium powder (0.37 g, 3.18 mmol, 3.0 equiv.) were dissolved in a mixture of EtOH (30 mL) and H_2O (2 mL). The resulting suspension was sonicated at 35–40 °C for 18 h, during the sonication process the indium, initially a fine powder, started to form grey lumps. The reaction mixture was conc. *in vacuo* and co-evaporated with toluene. EtOAc was added, the suspension was filtered over celite and washed with additional EtOAc. The filtrate was conc. *in vacuo*, affording an orange oil, which was purified by flash column chromatography (PE/EtOAc 1/0→19/1→9/1→4/1), affording compound **12** (0.6 g, 0.74 mmol, 70% over 2 steps, d.r. = ~3:1) as a partially separated mixture of diastereomers.* NMR signals and other experimental data of the major adduct **12a**: R_F = 0.22 (85/15 PE/Et_2O). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (dd, J = 12.4, 6.9 Hz, 4H, ArH), 7.48–7.14 (m, 16H), 6.08 (s, 1H), 5.90–5.70 (m, 1H), 5.39 (s, 1H), 5.35–5.16 (m, 2H), 5.16–4.88 (m, 3H), 4.88–4.67 (m, 1H), 4.41–4.36 (m, 1H), 4.15–4.10 (m, 2H), 4.10–4.04 (m, 2H), 4.02–3.89 (m, 1H), 3.81–3.67 (m, 2H), 3.57 (dd, J = 10.5, 7.2 Hz, 1H), 3.53–3.38 (m, 1H), 2.93 (t, J = 10.3 Hz, 1H), 1.36–1.30 (m, 3H), 1.28–1.14 (m, 5H), 1.10–1.02 (m, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5, 167.1, 158.6, 157.7, 139.8, 137.8, 137.6, 137.2, 136.3, 136.1, 136.0, 135.8, 135.8, 135.7, 134.7, 133.7, 129.8, 129.7, 129.6, 128.8, 128.7, 128.6, 128.5, 128.5, 128.4, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.3, 126.8, 120.6, 119.3, 107.8, 107.6, 78.7, 78.4, 75.7, 75.3, 75.0, 70.4, 68.5, 68.1, 67.8, 66.7, 63.0, 62.9, 61.2, 61.0, 60.4, 55.1, 48.4, 42.4, 41.8, 38.3, 37.5, 29.8, 27.1, 26.8, 26.7, 24.8, 19.3, 14.3, 14.2. FT-IR (neat): ν = 2932, 2858, 1731, 1697, 1497, 1456, 1427 cm^{-1}. HRMS calcd for $C_{48}H_{59}NO_8Si + Na^+$ [M + Na^+]: 828.3902. Found 828.3881. *NMR characterisation suffered from peak broadening and peak splitting due to the formation of rotamers. Minor compound **12b**: R_F = 0.14 (85/15 PE/Et_2O).

Benzyl benzyl((3R,4S)-3-((4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-5-hydroxy-7-(hydroxymethyl) octa-1,7-dien-4-yl)carbamate (13)

Diene ester **12** (27 mg, 34 μmol) was co-evaporated with anhydrous toluene (×3), dissolved in anhydrous toluene (1.5 mL) and cooled to −78 °C. A solution of DiBAl–H (1.0 M in hexane, 0.13 mL, 0.13 mmol, 3.8 equiv.) was added dropwise and the yellow solution was left to stir for 3 h. The reaction was quenched by adding sat. aq. Rochelle's salt (5 mL), the resulting suspension was allowed to warm to RT. The layers were separated, the organic layer was washed with brine (5 mL), dried with Na_2SO_4, filtered, and conc. *in vacuo*. The resulting yellow oil was purified using column chromatography (PE/EtOAc 1/0→95/5→9/1→4/1→3/2) to give the product **14** as a colourless oil (18 mg, 24 μmol, 69%).* R_F = 0.1 (85/15 PE/EtOAc). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.66 (m, J = 14.2, 7.1 Hz, 5H), 7.39 (m, 10H), 7.28 (m, 2H), 7.25–7.19 (m, 3H), 5.78 (dq, J = 14.2, 7.4, 4.9 Hz, 1H), 5.36–5.28 (m, 1H), 5.27–5.22 (m, 1H), 5.20 (d, J = 12.0 Hz, 1H), 5.12 (d, J = 14.6 Hz, 1H), 5.07–4.99 (m, 1H), 4.83 (s, 1H), 4.42 (s, 1H), 4.34 (m, 1H), 4.21 (d, J = 7.4 Hz, 1H), 4.12–4.05 (m, 1H), 3.99 (d, J = 8.41 1H), 3.89–3.80 (m, 2H), 3.78 (dt, J = 11.4, 5.2 Hz, 1H), 3.62 (dt, J = 31.2, 9.0 Hz, 2H), 3.47 (dt, J = 20.7, 7.3 Hz, 1H), 3.09 (dd, J = 11.4, 4.2 Hz, 1H), 1.60–1.52 (m, 1H), 1.47 (d, J = 20.7 Hz, 3H), 1.35 (s, 3H), 1.08 (d, J = 4.1 Hz, 9H), 1.00–0.94 (m, 1H). ^{13}C NMR (151 MHz, CDCl3) δ 139.80, 135.72, 135.67, 134.43, 133.48, 129.71, 129.62, 128.79, 128.71, 128.60, 128.51, 128.41, 128.32, 128.16, 127.85, 127.70, 127.61, 120.69, 119.07, 114.41, 113.73, 107.79, 78.49, 75.10, 70.32, 70.02, 68.24, 66.59, 66.28, 62.51, 54.64, 48.27, 41.37, 41.15, 29.65, 26.93, 26.52, 24.61. *NMR characterisation suffered from peak broadening and peak splitting due to the formation of rotamers.

Benzyl benzyl((1S,2R)-2-((4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-6-hydroxy-4-(hydroxymethyl) cyclohex-3-en-1-yl)carbamate (14)

Diene **14** was (7 mg, 9.2 μmol) was dissolved in anhydrous toluene (1.0 mL) after having been co-evaporated with the same solvent (× 3). A solution of Hoveyda-Grubbs catalyst C571 in toluene (0.01 M, 92 μL, 0.92 μmol, 10 mol%) was added and the mixture was heated

to 80 °C for 4 h. The mixture was directly purified by column chromatography (toluene/EtOAc 1:0→95:5→9:1→8:2) to give the cyclised product (**15**) as a green semisolid (4 mg, 5.4 μmol, 59%). R_F = 0.35 (7/3 Toluene/EtOAc). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.75–7.65 (m, 5H), 7.47–7.28 (m, 12H), 7.21–7.17 (m, 3H), 5.56 (s, 1H), 5.23 (d, J = 12.1 Hz, 1H), 5.09 (d, J = 12.3 Hz, 1H), 4.99 (d, J = 15.4 Hz, 1H), 4.38 (t, J = 6.6 Hz, 1H), 4.19–4.09 (m, 2H), 4.05 (s, 1H), 3.98–3.84 (m, 4H), 3.76 (s, 1H), 3.47–3.38 (m, 1H), 3.31 (s, 1H), 2.07–1.90 (m, 1H), 1.69–1.55 (m, 2H), 1.36 (s, 3H), 1.30 (s, 3H), 1.08 (s, 9H). ^{13}C NMR (101 MHz, cdcl3) δ 141.49, 136.01, 135.85, 133.38, 130.01, 129.87, 129.18, 128.78, 128.37, 127.94, 127.88, 127.77, 78.22, 74.94, 69.58, 68.12, 67.07, 62.27, 35.22, 33.97, 29.85, 29.43, 27.02, 26.63, 25.35, 21.60, 19.37. HRMS calcd for $C_{44}H_{53}NO_7Si + Na^+$ [M + Na^+]: 758.3484. Found 758.3514.

Benzyl benzyl((2R,3S)-2-((4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-4-hydroxy-6-(hydroxymethyl)-7-oxabicyclo[4.1.0]heptan-3-yl)carbamate (18)

Carbocycle **15** (3 mg; 4,1 μmol) was dissolved in DCM (1.0 mL) and cooled to 0 °C, *m*CPBA (~70%; 17 mg; 69 μmol; 17 equiv.) was added and the mixture was left to stir for 72 h. The mixture was added to a solution of sat. aq. $Na_2S_2O_3$ (1 mL), the layers were separated, and the aqueous layer was extracted with DCM (2× 2 mL). The organic layers were combined, dried with Na_2SO_4, filtered and concentrated under reduced pressure. Column chromatography (PE/EtOAc 1/0→9/1→4/1→3/2→1/1) of the residue gave the product **19** (2 mg, 2.6 μmol) as a colourless semisolid. R_F = 0.30 (1/1 PE/EtOAc). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.70–7.65 (m, 5H), 7.49–7.38 (m, 12H), 7.18 (d, J = 7.3 Hz, 3H), 5.18–5.08 (m, 2H), 4.62 (d, J = 18.9 Hz, 1H), 4.48 (d, J = 13.7 Hz, 1H), 4.38–4.32 (m, 1H), 4.32–4.26 (m, 1H), 4.06 (d, J = 6.8 Hz, 1H), 4.00 (d, J = 6.8 Hz, 1H), 3.93–3.86 (m, 1H), 3.78–3.67 (m, 3H), 3.66 (s, 1H), 3.49 (s, 2H), 2.32–2.28 (m, 1H), 1.68 (s, 2H), 1.50 (s, 3H), 1.33 (s, 3H), 1.09 (s, 9H).

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: Activity-based probes · Carbohydrates · Inhibitors · Sialic acids · Sialidases

[1] a) A. Varki, R. Schauer, in *Essentials of Glycobiology*. 2nd Ed. (Eds: A. Varki, R. D. Cummings, J. D. Esko) Cold Spring Harbor, New York, US **2009** Ch. 14; b) M. L. Huang, C. J. Fisher, K. Godula, *Exp. Biol. Med.* **2016**, *241*, 1042–1053.
[2] P. R. Crocker, J. C. Paulson, A. Varki, *Nat. Rev. Immunol.* **2007**, *7*, 255–266.
[3] V. Y. Glanz, V. A. Myasdoeva, A. V. Grechko, A. N. Orekhov, *Eur. J. Pharmacol.* **2019**, *842*, 345–350.
[4] A. Varki, *Trends Mol. Med.* **2008**, *14*, 351–360.
[5] B. L. Haines-Menges, W. B. Whitaker, J. B. Lubin, E. F. Floyd, *Microbiol. Spectr.* **2015**, DOI: 10.1128/microbiolspec.MBP-0005–2014.
[6] H. de Jong, M. M. S. M. Wösten, T. Wennekes, *Glycobiology* **2021**, DOI: 10.1093/glycob/cwab104.
[7] J.-H. Kim, R. Resende, T. Wennekes, H.-M. Chen, N. Bance, S. Buchini, A. G. Watts, P. Pilling, V. A. Streltsov, M. Petric, R. Liggins, S. Barret, J. L. McKimm-Breschkin, M. Niikura, S. G. Withers, *Science* **2013**, *340*, 71–75.
[8] S. Buchini, A. Buschiazzo, S. G. Withers, *Angew. Chem. Int. Ed.* **2008**, *47*, 2700–2703; *Angew. Chem.* **2008**, *120*, 2740–2743.
[9] D. J. Vocadlo, C. R. Bertozzi, *Angew. Chem. Int. Ed.* **2004**, *43*, 5338–5342.
[10] K. A. Stubbs, A. Scaffidi, A. W. Debowski, B. L. Mark, R. V. Stick, D. J. Vocadlo, *J. Am. Chem. Soc.* **2008**, *130*, 327–335.
[11] Y. M. C. A. Luikx, A. J. Henselijn, G. P. Bosman, D. A. T. Cramer, K. C. A. P. Giesbers, E. M. van't Veld, G.-J. Boons, A. J. R. Heck, K. R. Reiding, K. Strijbis, T. Wennekes, *Molecules* **2022**, *27*, 1615.
[12] a) C.-S. Tsai, H.-Y. Yen, M.-I. Lin, T.-I. Tsai, S.-Y. Wang, W.-I. Huang, T.-L. Hsu, Y.-S. E. Cheng, J.-M. Fang, C.-H. Wong, *PNAS* **2012**, *110*, 2466–2471; b) G. T. van der Horst, G. M. Mancini, R. Brossmer, U. Rose, F. W. Verheijen, *J. Biol. Chem.* **1990**, *265*, 10801–10804; c) C.-P Lu, C.-T Ren, Y.-N Lai, S.-H Wu, W.-M. Wang, J.-Y Chen, L.-C. Lo, *Angew. Chem. Int. Ed.* **2005**, *44*, 6888–6892.
[13] L. Wu, Z. Armstrong, S. P. Schröder, C. de Boer, M. Artola, J. M. F. G. Aerts, H. S. Overkleeft, G. J. Davies, *Curr. Opin. Chem. Biol.* **2019**, *53*, 25–36.
[14] J. Jiang, T. J. M. Beenakker, W. W. Kallemeijn, G. A. van der Marel, H. van den Elst, J. D. C. Codee, J. M. F. G. Aerts, H. S. Overkleeft, *Chem. Eur. J.* **2015**, *21*, 10861–10869.
[15] S. Vorwerk, A. Vasella, *Angew. Chem. Int. Ed.* **1998**, *37*, 1732–1734; *Angew. Chem.* **1998**, *110*, 1765–1767.
[16] J. Hoogenboom, M. Lutz, H. Zuilhof, T. Wennekes, *J. Org. Chem.* **2016**, *81*, 8826–8836.
[17] H. Lebel, V. Paquet, *J. Am. Chem. Soc.* **2004**, *126*, 320–328.
[18] a) S. Metthies, P. Stallforth, P. H. Seeberger, *J. Am. Chem. Soc.* **2015**, *137*, 2848–2851; b) S.-S. Chng, T.-G. Hoang, W.-W. W. Lee, M. P. Tham, H. Y. Ling, T. P. Loh, *Tet. Lett.* **2004**, *45*, 9501–9504; c) T.-H. Chan, M.-C. Lee, *J. Org. Chem.* **1995**, *60*, 4228–4232; d) M. D. Chappel, R. L. Halcomb, *Org. Lett.* **2000**, *2*, 2003–2005; e) J. Podlech, S. Steurer, *Eur. J. Org. Chem.* **1999**, 1551–1560.
[19] Y. Park, Y. Koga, C. Su, A. L. Waterbury, C. L. Johnny, B. B. Liau, *Angew. Chem. Int. Ed.* **2019**, *58*, 5387–5.

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