^{19}F NMR-Guided Design of Glycomimetic Langerin Ligands

Eike-Christian Wamhoff[1,2], Jonas Hanske[1,2], Lennart Schnirch[2], Jonas Aretz[1,2], Maurice Grube[1,2], Daniel Varón Silva[1,2] and Christoph Rademacher[1,2]*

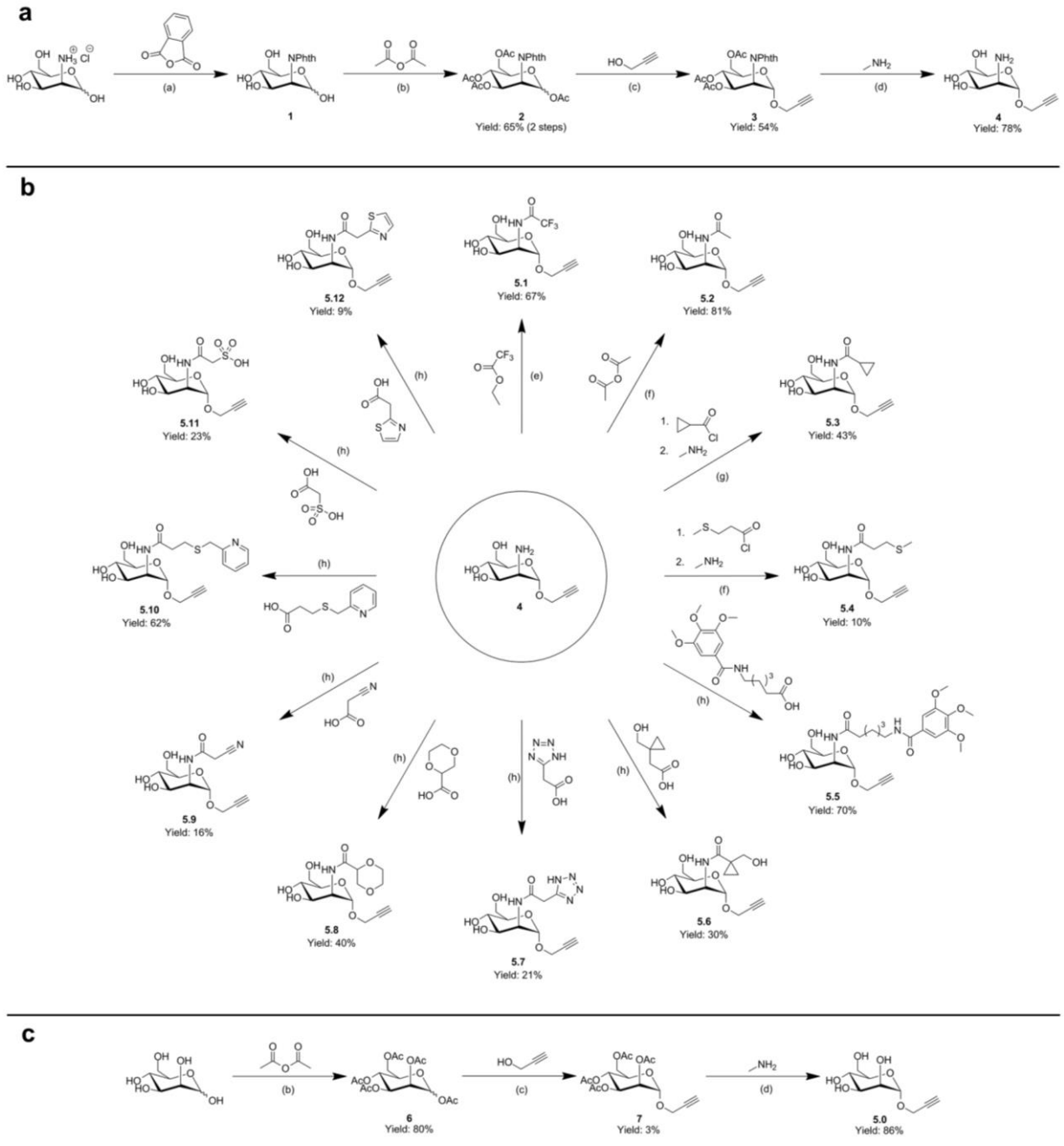
[1]Max Planck Institute of Colloids and Interfaces, Department of Biomolecular Systems, 14424 Potsdam, Germany

[2]Freie Universität Berlin, Department of Biology, Chemistry and Pharmacy, 14195 Berlin, Germany

Supporting Information

I. Supporting Figures, Tables and Schemes

Scheme S1. Synthesis of 2-carboxamido-2-deoxy-α-mannoside and α-mannoside analogs 5. a. Reaction conditions for the preparation of precursor **4**: (a) acetone:H_2O (1:1), 50°C; (b) pyridine, 50°C; (c) $BF_3{\cdot}OEt_2$, anhydrous DCM:ether (2:1), 0°C to room temperature; (d) EtOH, room temperature. **b.** Reaction conditions for the preparation of 2-carboxamido-2-deoxy-α-mannoside analogs **5**: (e) MeOH, room temperature; (f) DMF, room temperature; (g) pyridine, room temperature; (h) PyBOP, DIPEA, DMF, room temperature. **c.** Reaction conditions for the preparation of α-mannoside analog **5.0**.



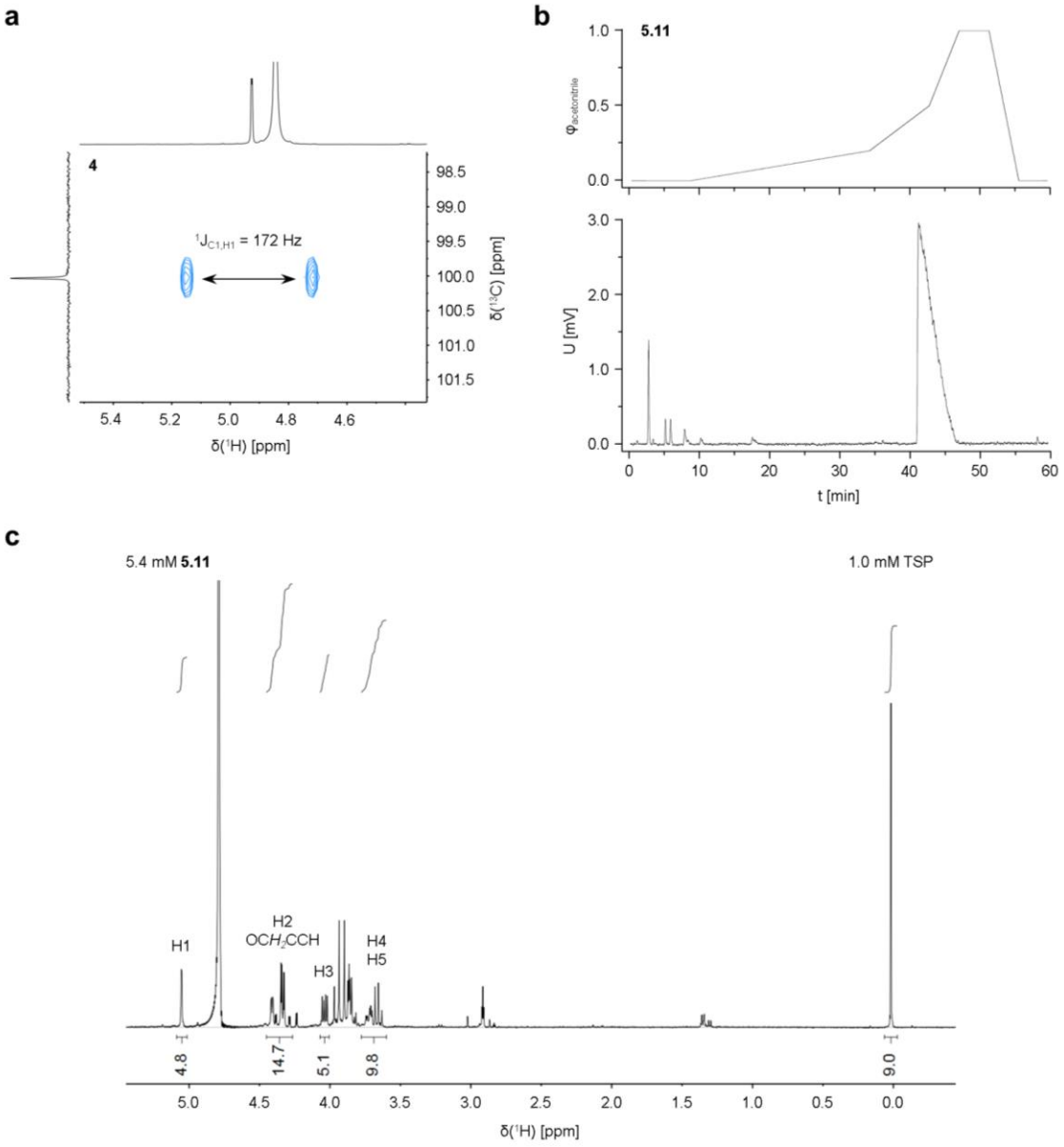


Figure S1. Supporting information for the synthesis of 2-carboxamido-2-deoxy-α-mannoside analogs 5. a. The α-configuration of precursor **4** was validated via the determination of the coupling constant $^1J_{C1,H1}$ from ^{13}C HSQC NMR spectra (*1*). **b.** The purity of **5.11** was analyzed via analytical HPLC run on a HyperCarb column using a 0.1% FA in H_2O-acetonitrile gradient, a flow rate of 1.0 ml·min^{-1} and ELSD. **c.** ^{1}H NMR experiments in presence of TSP in D_2O revealed a purity of 89% to 94% for **5.11**, depending on the integrated resonances.

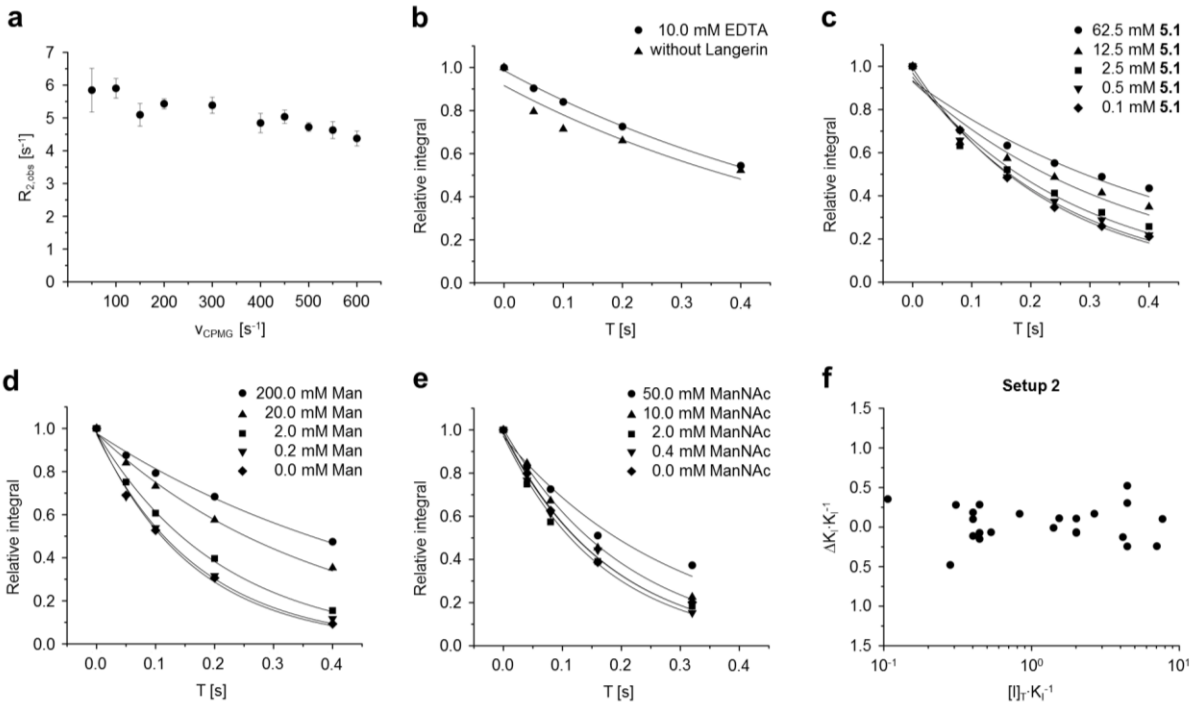


Figure S2. Supporting information for the ^{19}F R_2-filtered NMR assay. a. Relaxation dispersion experiments at 0.1 mM **5.1** in presence of the Langerin ECD indicate a negligible exchange contribution $R_{2,ex}$ at a ν_{CPMG} value of 500 Hz. **b.** The decay curve at 12.5 mM **5.1** in presence of the ECD and EDTA is depicted. The comparison with a representative decay curve at 0.1 mM **5.1** in absence of Langerin validates the Ca^{2+}-dependency of the interaction. **c.** The decay curves for the titration with **5.1** in presence of the CRD are shown. **d. and e.** Representative decay curves from the competitive binding experiments with Man and ManNAc are shown. **f.** 23 data points selected from competitive binding experiments with **5.7**, **5.8**, **5.9**, **5.11**, Man and ManNAc served to simulate the assay performance in Setup 2.

Table S1. Supporting information for the ^{19}F R_2-filtered NMR assay.

	ECD	CRD
$R_{2,f}$ [s^{-1}]	1.8±0.2[a]	1.8±0.2[a]
$R_{2,b}$ [s^{-1}]	660.3±48.2[b]	361.5±46.5
K_D [mM]	7.9±0.7[b]	7.3±1.0

[a]n = 4.
[b]n = 3.

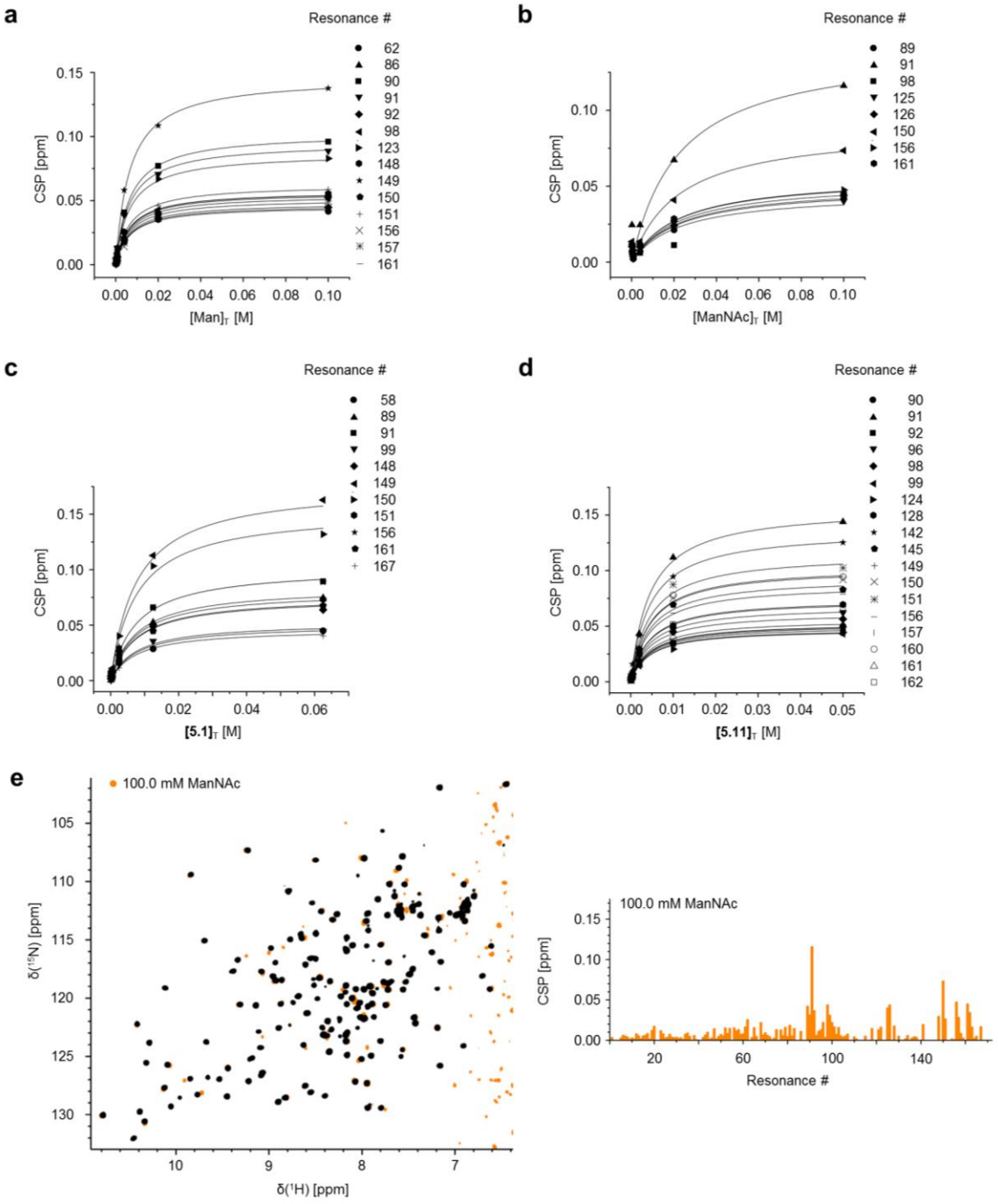


Figure S3. Supporting information for the ^{15}N HSQC NMR assay. a. to d. Resonances with CSP values higher than 0.04 ppm at the highest ligand concentration were selected for the determination of the K_D values of Man, ManNAc, **5.1** and **5.11**. **e.** The CSP fingerprint observed for ManNAc suggests a binding mode similar to that of Man, **5.1** and **5.11**.

Table S2. Comparison of CSP fingerprints for Man, 5.1 and 5.11. For all resonances that display a CSP higher than 0.04 ppm in presence of 100.0 mM Man, CSPs are also observed upon titration with 62.5 mM **5.1** or 50.0 mM **5.11**. These findings suggest a similar binding mode.

	CSP [ppm]		
Resonance #	100.0 mM Man	62.5 mM **5.1**	50.0 mM **5.11**
62	0.041	0.029	0.039
86	0.055	0.025	0.027
90	0.096	0.022	0.050
91	0.089	0.090	0.144
92	0.045	0.023	0.045
98	0.052	0.020	0.056
123	0.083	n.d.*	0.020
148	0.055	0.064	0.050
149	0.138	0.163	0.139
150	0.053	0.132	0.092
151	0.059	0.073	0.102
156	0.044	0.089	0.079
157	0.050	0.010	0.049
161	0.054	0.068	0.051

*resonance not found in reference spectrum

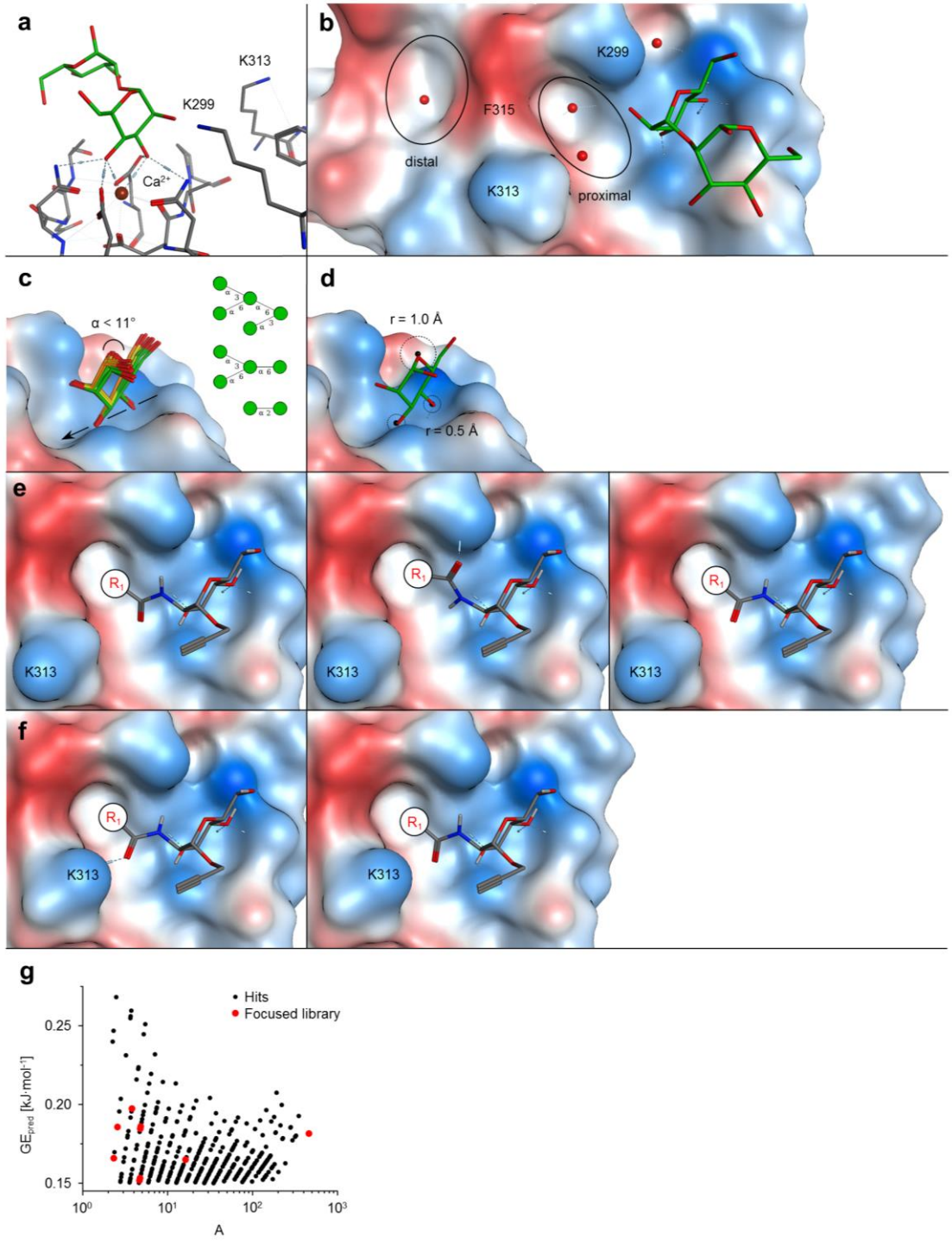


Figure S4. Structure-based *in silico* design of 2-carboxamido-2-deoxy-α-mannoside analogs 5. a. Man recognition by Langerin is driven by the Ca^{2+}-coordination via two equatorial hydroxyl groups. Only few secondary interactions are observed (*2*). **b.** The surface representation reveals two pockets in axial direction of C2 of the Man scaffold. Hydrophilic regions of the receptor surface are depicted in blue while hydrophobic regions are depicted in red. **c.** A structural alignment of the binding sites of available X-ray structures of Langerin in complex with different oligomannosides is depicted (3P5D.pdb, 3P5E.pdb and 3P5F.pdb) (*2*). The orientation of the directly bound Man is highly conserved. **d.** A pharmacophore model was defined to constrain the orientation of the Man scaffold during the force field-based refinement of generated docking poses. All features displayed require an oxygen atom within the indicated spheres. **e. and f.** Different amide linker conformations were selected from low mode molecular dynamics simulations and utilized for the *in situ* conjugation of commercially available carboxylic acids. Moreover, the alternative conformation of K313 observed for the complex with Gal-6S was accounted for in additional docking runs (*2*). **g.** The distribution of hits with respect to the predicted affinity increase A and the corresponding group efficiency GE_{pred} is depicted. The focused library of analogs **5** was selected from hits displaying a GE_{pred} value higher than 0.15 kJ·mol^{-1}.

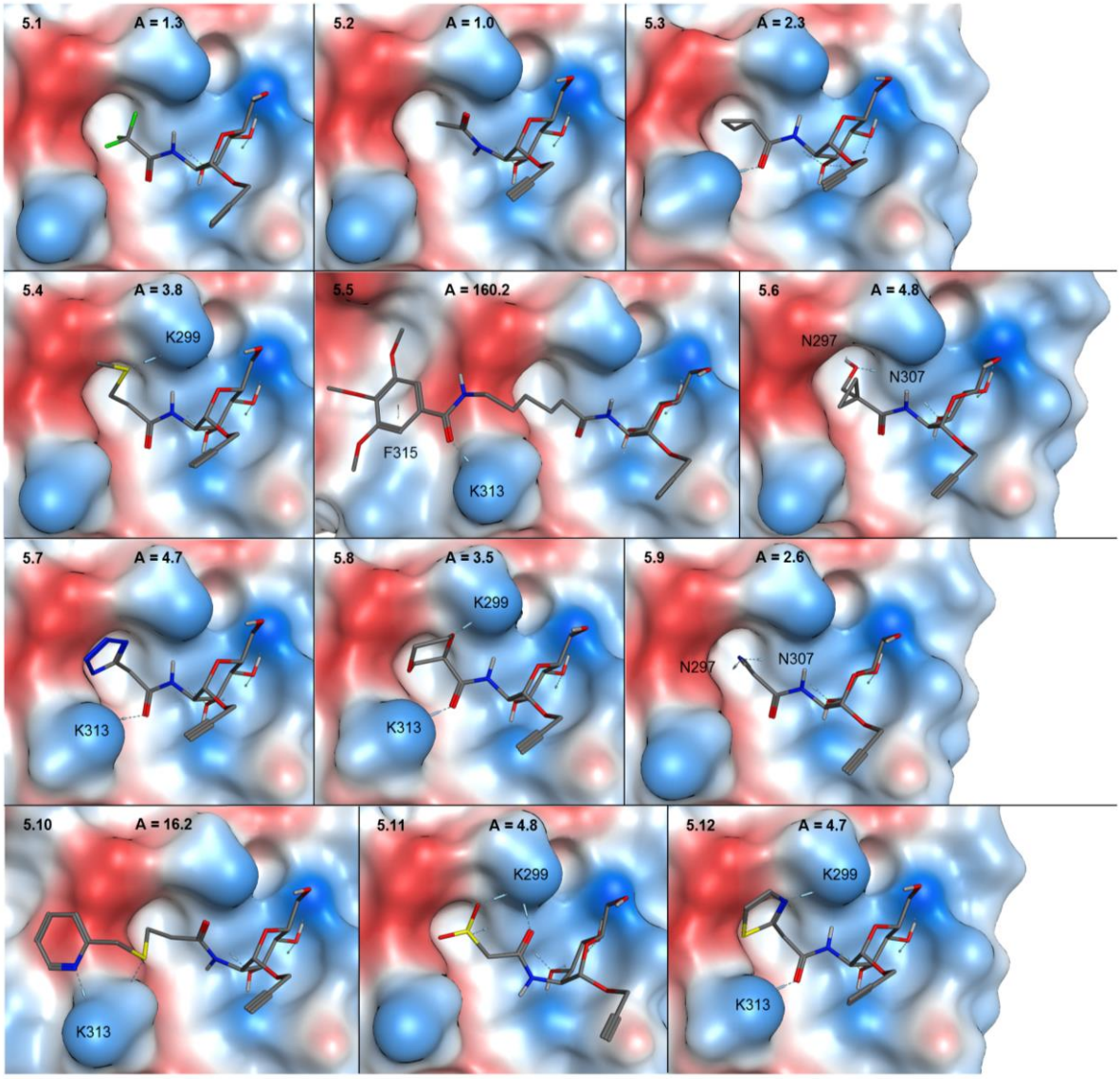


Figure S5. Docking poses for 2-carboxamido-2-deoxy-α-mannoside analogs 5. For each analog **5** the docking pose with the highest GBVI/WSA ΔG score is depicted. The corresponding scores served to predict the affinity increase over **5.2**. Residues involved in directed interactions with the substituents in C2 of the Man scaffold are indicated. Hydrophilic regions of the receptor surface are depicted in blue while hydrophobic regions are depicted in red.

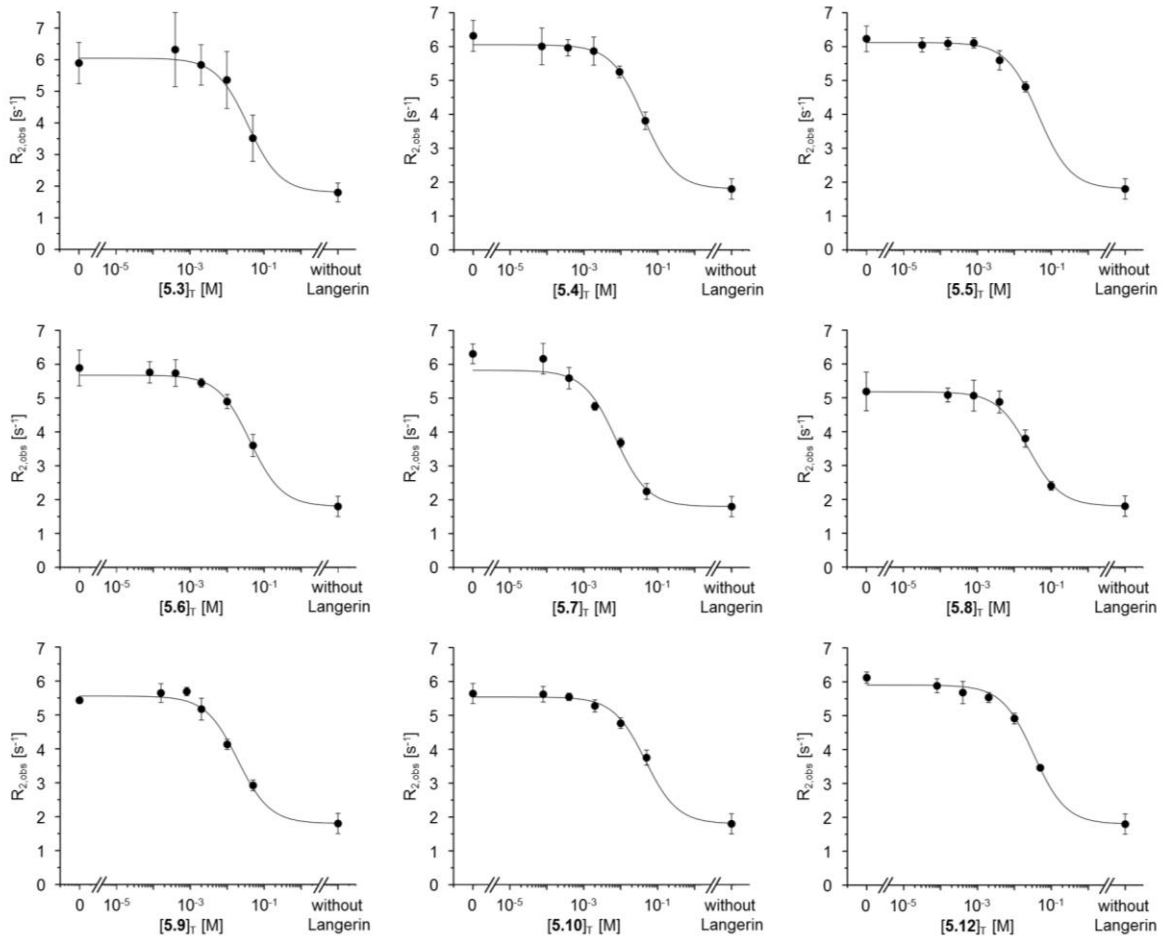


Figure S6. Determination of K_I values for 2-carboxamido-2-deoxy-α-mannoside analogs 5. Equation 3 was fitted to recorded $R_{2,obs}$ values to determine $[P]_T$ and K_I values. The results are summarized in Table 1.

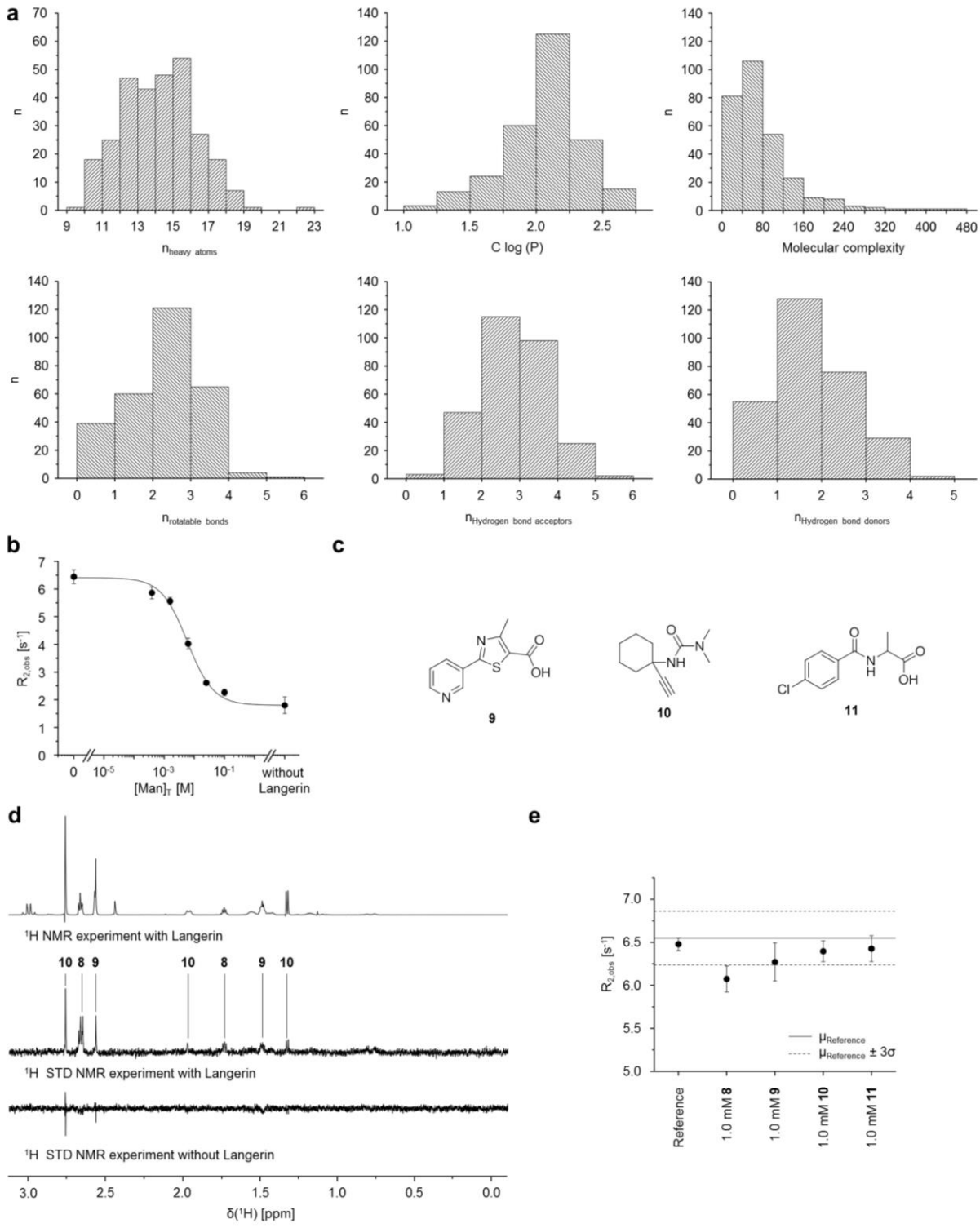


Figure S7. Supporting information for the explorative fragment screening. a. 290 fragments were randomly selected from our in-house fragment library and screened against Langerin with the ^{19}F R_2-filtered NMR assay. Relevant descriptors such as C log (P), molecular complexity as well as the number of heavy atoms, non-terminal rotatable bonds, hydrogen bond acceptors and hydrogen bond donors are compliant with published guidelines for fragment library design (*3-5*). **b.** Fragment mixtures were screened in presence of 10% DMSO and 0.01% Tween-20. The determined K_I value of 5.6±0.2 mM for Man in presence of these additives is comparable to the affinity obtained in their absence. **c. and d.** The fragment mixture displaying the highest inhibition was analyzed by ^{1}H STD NMR. Fragments **8**, **9**, **10** and **11** were found to interact with Langerin. STD spectra are magnified 25-fold. **e.** Upon deconvolution of this mixture, only fragment **8** was observed to compete with reporter molecule **5.1**.

II. Methods

Molecular Modelling

General remarks

Molecular modelling procedures were performed in MOE (*6*). Unless stated otherwise, options and parameters were set to default. The AMBER10:EHT force field was selected for the refinement of docking poses and the hydrogen bond network while MMFF94x was utilized for the generation of carboxylic acid conformers (*7-9*). Databases were processed in KNIME and tautomers were enumerated with ChemAxon's Calculator Plugin (*10*).

Definition of the pharmacophore model and preparation of the Langerin complex

A structural alignment of Langerin carbohydrate binding sites of in complex with different oligomannosides was performed (3P5D.pdb, 3P5E.pdb and 3P5F.pdb) (*2*). Based on this visualization, a pharmacophore model was defined with features for O3, O4 and O5 of the Man scaffold. The spatial constraint on the O3 and O4 was defined by a sphere with a radius r of 0.5 Å while the position of O5 was constrained by a sphere with a radius r of 1.0 Å. Chain B of the Langerin CRD in complex with a dimannoside served as the structural basis for the performed *in silico* screening (3P5F.pdb). Of the two binding modes included in this model, the orientation for targeting the identified pockets in axial direction of C2 was selected. Additionally, an alternative conformation for K313 observed for the Langerin complex with Gal-6S was modeled and included into the analysis (*11*). Overall model quality and protein geometry were evaluated in MolProbity (*12*). Next, protonation states and the hydrogen bond network of the complex were simulated with MOE's Protonate 3D followed by the removal of all solvent molecules. Subsequently, a propargyl group was modeled to the anomeric position of the mannose scaffold and the axial hydroxyl group in C2 was substituted with an acetamido group. The conformational space for the dihedral angle of the C2-N bond was explored in context of the binding site via Low Mode MD simulations assuming trans configuration of the acetamido group (*13*). Five energetically favorable rotamers corresponding to two different conformations of K313 were identified and served as the structural basis for the subsequent *in silico* screening of the carboxylic acid conformation database against Langerin.

Preparation of the carboxylic acid conformation database

Carboxylic acid conformations were generated from building block databases of selected manufacturers (TimTec, TCI, Sigma Aldrich, Otava Chemicals, Life Chemicals, Focus Synthesis, Enamine, ChemDiv, ChemBridge and Asinex). The database was processed with MOE's Wash and

filtered to yield structures with a maximum of 23 heavy atoms and 6 rotatable bonds. Moreover, only molecules containing one carboxyl group and no amino, azido or alkyne group were retained. Next, this subset was filtered for reactive molecules followed by the generation of tautomers and protonation states with ChemAxon's Calculator Plugin (*14*, *15*). Subsequently, the carboxyl group was removed and substituted by an annotated atom with MOE's Combinatorial Library that served as an annotation point for virtual conjugation to the modified Man scaffold *in situ*.

In silico **screening of the conformation database against Langerin**

The docking procedure was implemented with MOE's Combinatorial Builder. The carboxylic acids were conjugated to the modified Man scaffold by substituting the terminal methyl group via the virtual formation of a C-C bond. A grid-based placement method was utilized for generating docking poses by exploring the conformational space around this bond. During the subsequent force field-based structure refinement, the binding mode of the mannose scaffold was constrained by the pharmacophore model described above. Conformational flexibility of the binding site was accounted for by introducing B-factor-derived tethers to side chain atoms. Refined docking poses were then filtered by the pharmacophore model, scored with the GBIV/WSA ΔG function and written into the output database. Next, scores were referenced against **5.2** and calculated GBIV/WSA ΔΔG values served to determine the predicted group efficiency GE_{pred} as well as the predicted affinities increase A. Only poses with a GE_{pred} value higher than 0.15 $kJ \cdot mol^{-1}$ and an RMSD upon refinement lower than 2 Å were retained. Highly scored 2-deoxy-2-carboxamido-α-mannoside analogs **5** were evaluated visually and a focused library was composed. The composition of the library was guided by an attempt to maximize the diversity of pharmacophore features and to ensure synthetic feasibility. Importantly, this first generation of analogs was selected to test basic binding hypotheses and to establish a structure activity relationship in axial direction of the C2.

Receptor Expression and Purification

General remarks

Codon-optimized genes for the expression of Langerin in *E. coli* were purchased from GenScript. All growth media or chemicals used for receptor expression and purification were purchased from Carl Roth if not stated otherwise.

Langerin ECD

The truncated Langerin ECD (residues 148 to 328, forward primer: GGTGGTCATATGGCCTCGAC GCTGAATGCCCAGATTCCGG, reverse primer: ACCACCAAGCTTTTATTTTTCAAACTGCGG ATG) was cloned with a C-terminal TEV cleavage site and a Strep-tag II into a pET30a expression vector (EMD Millipore) and expressed insolubly in *E. coli* BL21* (DE3) (Invitrogen). Precultures were incubated overnight in LB medium supplemented with 35 µg·ml^{-1} Kanamycin (50 ml) at 37° C and 220 rpm. The preculture was diluted to an OD_{600} of 0.1 into LB medium supplemented with 35 mg·ml^{-1} Kanamycin (500 ml). The culture was incubated at 37° C and 220 rpm and expression of the Langerin ECD was induced with 0.5 mM IPTG at an OD_{600} of 0.6 to 0.8. Cells were harvested 4 h after induction via centrifugation at 4000 g and 4° C for 20 min. Cell pellets were stored overnight at -20° C and subsequently resuspended in 50 mM Tris with 0.1% Triton X-100 and 10 mM $MgCl_2$ (20 ml) at pH 7.5. Lysozyme (Sigma Aldrich) was added and the sample was incubated for 3.5 h at 4° C. After the addition of DNase I (AppliChem) the sample was incubated for another 30 min at 4° C. Inclusion bodies were harvested via centrifugation at 10000 g and 4° C for 10 min and washed three times with 25 mM Tris with 150 mM NaCl (20 ml) at pH 7.8. Inclusion body pellets were stored overnight at -20° C and subsequently solubilized overnight in 100 mM Tris with 6 M Gu-HCl and 1 mM DTT (20 ml) at pH 8.0 and 30° C. Following centrifugation at 15000 g and 4°C for 1.5 h, the Langerin ECD was refolded overnight via rapid dilution into 50 mM Tris with 0.4 M arginine, 20 mM NaCl, 0.8 mM KCl, 1 mM glutathione (AppliChem) and 0.2 mM glutathione disulfide (AppliChem) (200 ml) at pH 7.6 and 4°C. Next, the sample was dialyzed overnight against 25 mM Tris, 150 mM NaCl, 25 mM $CaCl_2$ at pH 7.8 and 4° C. After centrifugation at 15000 g and 4° C for 2 h, the sample was purified as via mannan-agarose (Sigma Aldrich, St. Louis, USA) affinity chromatography as previously published (*16*). The buffer was exchanged to 25 mM Tris with 150 mM NaCl at pH 7.8 via 7 kDa size-exclusion desalting columns (Thermo Scientific) and the concentration of Langerin ECD was determined via UV spectroscopy ($A_{280,\ 0.1\%}$ = 2.45) (*17*). Typical yields were in the range are of 10 mg·l^{-1} bacterial culture. Purity and monodispersity of Langerin ECD samples were analyzed via SDS PAGE and DLS, respectively.

Langerin CRD

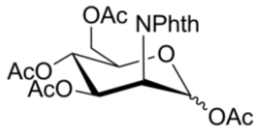
The Langerin CRD (residues 193 to 328, forward primer: GGTGGTCATATGGCCCAGGTGGTTAG CCAAGGCTGGAAATAC, reverse primer: ACCACCAAGCTTTTATTTTTCAAACTGCGGATG) was cloned with a C-terminal TEV cleavage site and a Strep-tag II into a pET30a expression vector (Invitrogen) and expressed insolubly in *E. coli* BL21* (DE3) (Invitrogen). Precultures were incubated overnight in M9 medium supplemented with 35 µg·ml^{-1} Kanamycin and ^{15}N-labeled NH_4Cl (Sigma Aldrich) (50 ml) at 37° C and 220 rpm. The preculture was diluted to an OD_{600} of 0.1 into M9 medium supplemented with 35 mg·ml^{-1} Kanamycin and ^{15}N-labeled NH_4Cl (Sigma Aldrich) (500 ml). The culture was incubated at 37° C and 220 rpm and expression of the Langerin CRD was induced with 0.5 mM IPTG at an OD_{600} of 0.6 to 0.8. Cells were harvested 4 h after induction via centrifugation at 4000 g and 4° C for 20 min. Cell pellets were stored overnight at -20° C and subsequently resuspended in 50 mM Tris with 0.1% Triton X-100 and 10 mM $MgCl_2$ (20 ml) at pH 7.5. Lysozyme (Sigma Aldrich) was added and the sample was incubated for 3.5 h at 4° C. After the addition of DNase I (AppliChem) the sample was incubated for another 30 min at 4° C. Inclusion bodies were harvested via centrifugation at 10000 g and 4° C for 10 min and washed three with 25 mM Tris with 150 mM NaCl (20 ml) at pH 7.8. Inclusion body pellets were stored overnight at -20° C and subsequently solubilized overnight in 100 mM Tris with 6 M Gu-HCl and 1 mM DTT (20 ml) at pH 8.0 and 30° C. Following centrifugation at 15000 g and 4°C for 1.5 h, the Langerin CRD was refolded overnight via rapid dilution into 50 mM Tris with 0.8 M arginine, 20 mM NaCl, 0.8 mM KCl, 1 mM glutathione (AppliChem) and 0.2 mM glutathione disulfide (AppliChem) (200 ml) at pH 7.6 and 4°C. Next, the sample was dialyzed overnight against 50 mM Tris, 150 mM NaCl, 1 mM EDTA at pH 8.0 and 4° C. After centrifugation at 15000 g and 4° C for 2 h, the sample was purified as via StrepTactin affinity chromatography (Iba). The Langerin CRD was eluted with 50 mM Tris with 2.5 mM d-desthiobiotin, 150 mM NaCl at pH 7.5 and dialyzed against 25 mM MES with 40 mM NaCl at pH 6.0. After centrifugation at 15000 g and 4° C for 1.5 h, the buffer was exchanged to 25 mM HEPES with 150 mM NaCl at pH 7.0 via 7 kDa size-exclusion desalting columns (Thermo Scientific) and the concentration of Langerin CRD was determined via UV spectroscopy ($A_{280,\ 0.1\%}$ = 3.19) (*17*). Typical yields were in the range are of 5 mg·l^{-1} bacterial culture. Purity and monodispersity of Langerin CRD samples were analyzed via SDS PAGE and DLS, respectively.

Synthetic Chemistry

General remarks

Reagents and solvents used were purchased from Sigma Aldrich unless indicated otherwise and used as supplied without any further purification. Anhydrous solvents were taken from an anhydrous solvent system (JC-Meyer Solvent Systems). Column chromatography was carried out using silica gel at a pore size from 40 to 60 Å (Machery Nagel). Reversed-phase column chromatography was carried out using Chromabond endcapped C_{18} columns at a pore size of 60 Å (Machery Nagel). Analytical TLC was performed on glass plates coated with silica gel at a pore size of 60 Å (Machery Nagel). Compounds were detected via 3-methoxyphenol reagent (0.2% 3-methoxyphenol in EtOH: 2 N sulfuric acid in EtOH (1:1)), ninhydrin reagent (1.5 g ninhydrin in 15 ml acetic acid and 500 ml MeOH) or CAM reagent (1.0 g $Ce(SO_4)_2 \cdot 4H_2O$ and 2.5 g ammonium molybdate pentahydrate in 96 ml of H_2O and 6 ml of concentrated H_2SO_4) upon heating or via UV adsorption (λ = 254 nm). NMR experiments were conducted on a OneNMR 400 MHz or 600 MHz spectrometer (Agilent). Chemical shifts were referenced to the internal standards $CHCl_3$ ($\delta(^1H)$ = 7.26 ppm and $\delta(^{13}C)$ = 77.1 ppm), H_2O ($\delta(^1H)$ = 7.26 ppm), MeOH ($\delta(^1H)$ = 4.87 ppm, $\delta(^{13}C)$ = 49.0 ppm) and trifluoroacetic acid ($\delta(^{19}F)$ = 76.55 ppm). Coupling constants are reported in Hz and coupling patterns are indicated as s for singlet, d for doublets, dd for doublets of doublets, ddd for doublets of doublets of doublets, t for triplets, dt for doublets of triplets, td for triplet of doublets, q for quartets and m for multiplets. Signals were assigned by means of COSY, TOCSY and ^{13}C HSQC NMR experiments. Stereoselectivity at the anomeric position of the mannose scaffold was analyzed by measuring $^1J_{C1,H1}$ coupling constants for **3**, **4** and **7** (*18*). NMR spectra were processed with MestReNova (*19*). ESI-MS analysis was conducted using an 1100 Series LC/MS coupled to a G1946D ESI-Q spectrometer (Agilent). HR ESI-MS analysis was conducted using a 6210 ESI-TOF spectrometer (Agilent) or an Acquity H-Class UPLC/MS coupled to a Xevo G2-S ESI-Q-TOF spectrometer (Waters). Analytical HPLC was performed on a 1200 Series LC/MS coupled to a 6130 ESI-Q spectrometer (Agilent) using an analytical HyperCarb column (Thermo Scientific). Preparative HPLC was performed on a 1200 Series LC/MS using a semi-preparative HyperCarb column (Thermo Scientific).

1,3,4,6-tetra-O-acetyl-2-deoxy-2-phthalimido-D-mannopyranose



2

2 was prepared as previously published (*20*). Mannosamine hydrochloride (Dextra) (2.15 g, 10 mmol), phthalic anhydride (1.63 g, 11 mmol) and pyridine (2 ml, 25 mmol) were dissolved in a mixture of acetone-H_2O (1:1, 15 ml) and stirred at 50°C for 3 h. Progress of the reaction was monitored by analytical TLC (propan-1-ol: ethyl acetate: H_2O: 25% aqueous ammonia (6:3:1:1)). Solvents were evaporated *in vacuo* and acetic anhydride (14.1 ml, 150 mmol) and pyridine (40 ml) were added to the residue. The mixture was heated to 50°C and stirred for 5 h. Progress of the reaction was monitored by analytical TLC (toluene: ethyl acetate (4:1)). Solvents were evaporated *in vacuo* and the residue was taken up in chloroform (250 ml). The organic phase was extracted with 1 M HCl, saturated $NaHCO_3$ and H_2O. Subsequently, the organic phase was dried with $MgSO_4$. Solvents were evaporated *in vacuo* and the residue was purified via column chromatography (toluene: ethyl acetate (8:1)) to afford an α/β-anomer mixture of **2** (3.10 g, 6.50 mmol, 65 %) as a white solid.

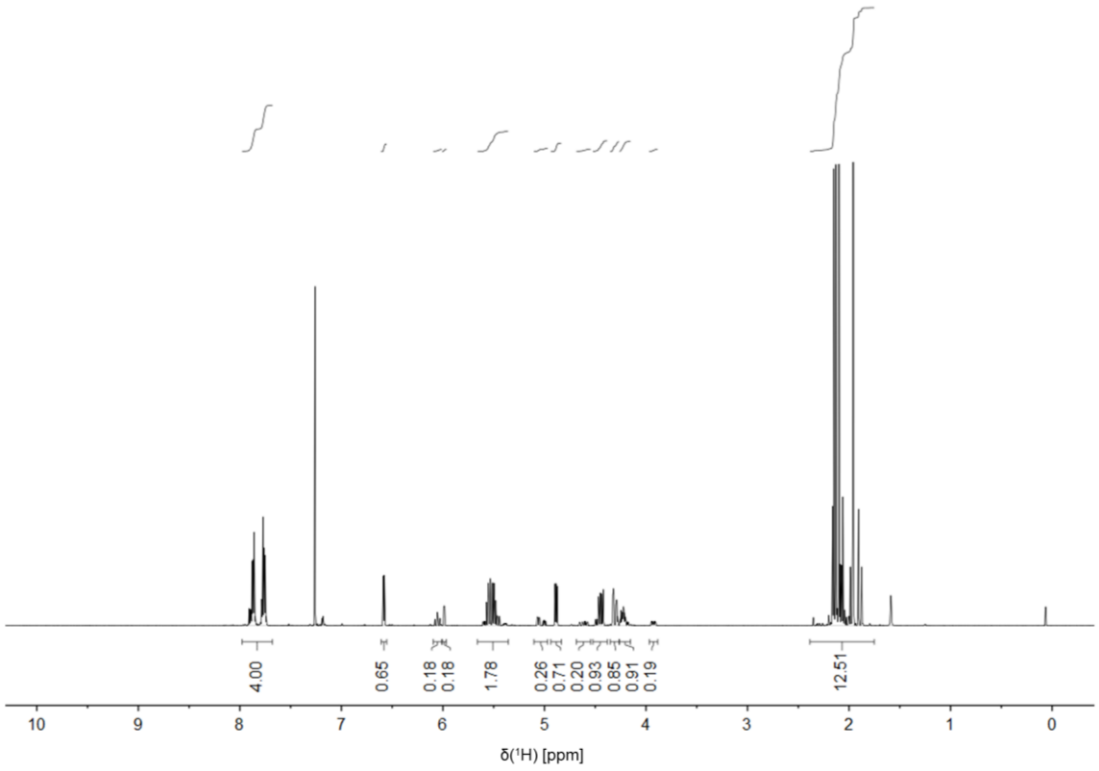
^{1}H NMR (400.0 MHz, $CDCl_3$, α-anomer): δ = 7.92 - 7.75 ppm, m, 4 H (aromatic H of Phth); δ = 6.59 ppm, d, 1 H (H1); δ = 5.55 ppm, dd, 1 H, J = 6.8, 8.2 Hz (H4); δ = 5.50 ppm, dd, 1 H, J = 5.3, 6.8 Hz (H3); δ = 4.89, dd, 1H, J = 3.9, 5.3 Hz (H2); δ = 4.45 ppm, dd, 1 H, J = 6.0, 12.2 Hz, (H6a); δ = 4.31, dd, 1 H, J = 3.3, 12.2 ppm (H6b); δ = 4.23 ppm, ddd, 1 H, J = 3.3, 6.0, 8.3 Hz, (H5);δ = 2.15 ppm, s, 3 H ($OCOCH_3$); δ = 2.13 ppm, s, 3 H ($OCOCH_3$); δ = 2.10 ppm, s, 3 H ($OCOCH_3$); δ = 1.96 ppm, s, 3 H ($OCOCH_3$).

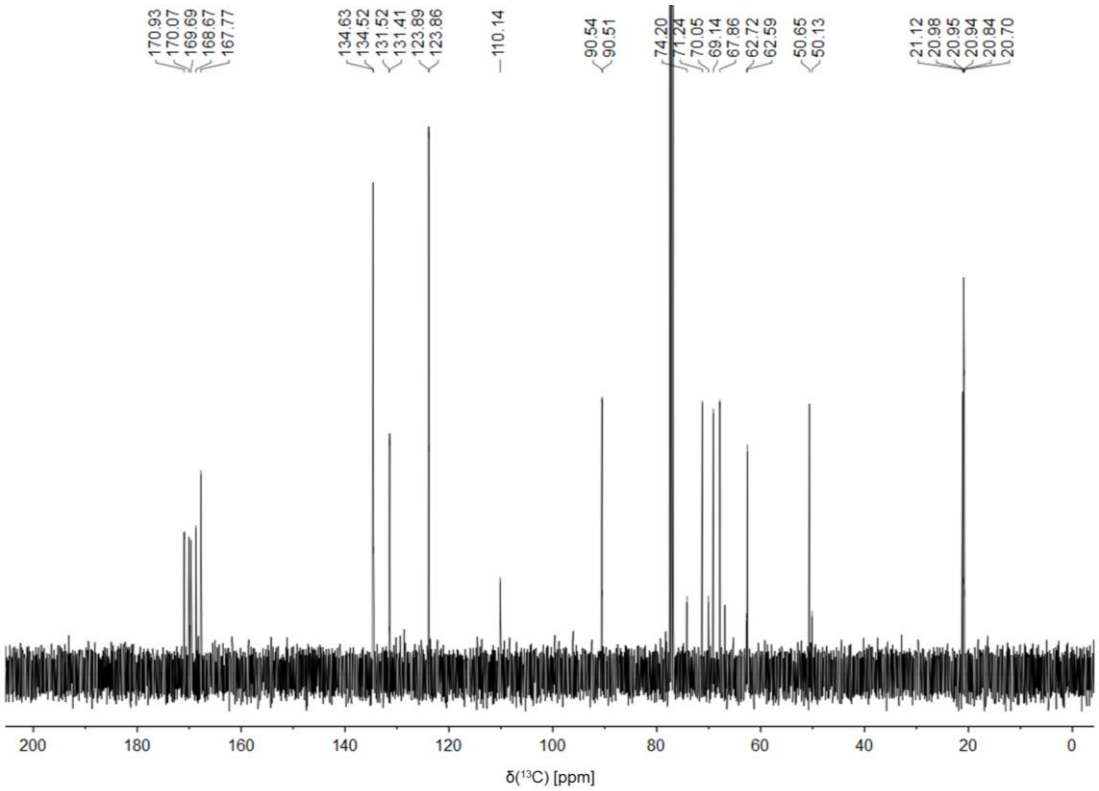
^{13}C NMR (100.6 MHz, $CDCl_3$, α-anomer): δ = 170.8 ppm, 1C ($OCOCH_3$); δ = 169.9, 1C ($OCOCH_3$); δ = 169.5 ppm ($OCOCH_3$); δ = 168.5 ppm, 1C ($OCOCH_3$); δ = 167.6 ppm, 2 C (carbonyl C of Phth); δ = 134.5 ppm, 2 C (aromatic C of Phth); δ = 131.3 ppm, 2 C (aromatic C of Phth); δ = 123.8 ppm, 2 C (aromatic C of Phth); δ = 90.4 ppm, 1 C (C1); δ = 71.1 ppm, 1 C (C5); δ = 69.0 ppm, 1 C (C3); δ = 67.8 ppm, 1 C (C4); δ = 62.5 ppm, 1 C (C6); δ = 50.5, 1 C (C2); δ = 21.0 ppm, 1 C ($OCOCH_3$); δ = 20.8 ppm, 2 C (two times $OCOCH_3$); δ = 20.7 ppm, 1 C ($OCOCH_3$).

^{1}H NMR (400.0 MHz, $CDCl_3$, β-anomer): δ = 7.92 - 7.75 ppm, m, 4 H (aromatic H of Phth); δ = 6.06 ppm, t, 1 H, J = 9.5, 9.5 Hz (H4); δ = 5.99 ppm, d, 1 H, J = 2.8 Hz (H1); δ = 5.37 ppm, dd, 1 H, J = 6.8, 9.4 Hz (H3); δ = 5.06 ppm, dd, J = 2.8, 6.8 Hz (H2); δ = 4.48 ppm, dd, 1 H, J = 6.0 ppm, 12.2 (H6a); δ = 4.27, dd, 1 H, J = 2.3, 12.2 ppm (H6b); δ = 3.93 ppm, ddd, 1 H, J = 2.3, 6.0, 9.7 (H5); δ = 2.16 ppm, s, 3 H ($OCOCH_3$); δ = 2.07 ppm, s, 3 H ($OCOCH_3$); δ = 1.97 ppm, s, 3 H ($OCOCH_3$); δ = 1.91 ppm, s, 3 H ($OCOCH_3$).

^{13}C NMR (100.6 MHz, $CDCl_3$, β-anomer): δ = 170.7 ppm, 1 C ($OCOCH_3$); δ = 169.7, 1 C ($OCOCH_3$); δ = 169.5 ppm, 1 C (OCOCH3); δ = 168.4 ppm, 1 C ($OCOCH_3$); δ = 168.0 ppm, 2 C (carbonyl C of Phth); δ = 134.3 ppm, 2 C (aromatic C of Phth); δ = 131.2 ppm, 2 C (aromatic C of Phth); δ = 123.6 ppm, 2 C (aromatic C of Phth); δ = 90.3 ppm, 1 C (C1); δ = 73.9 ppm, 1 C (C5); δ = 69.8 ppm, 1 C (C3); δ = 66.7 ppm, 1 C (C4); δ = 62.5 ppm, 1 C (C6); δ = 49.9, 1 C (C2); δ = 20.7 ppm, 2 C (two times $OCOCH_3$); δ = 20.5 ppm, 1 C ($OCOCH_3$); δ = 20.4 ppm, 1 C ($OCOCH_3$).

R_f = 0.35 with toluene:ethyl acetate (2:1).

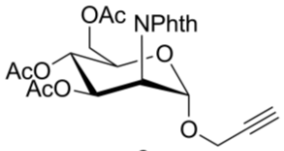



170.93
170.07
169.69
168.67
167.77
134.63
134.52
131.52
131.41
123.89
123.86
110.14
90.54
90.51
74.20
71.24
70.05
69.14
67.86
62.72
62.59
50.65
50.13
21.12
20.98
20.95
20.94
20.84
20.70
200
180
160
140
120
100
80
60
40
20
0
δ(13C) [ppm]

271

272

Propargyl-3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-α-D-mannopyranoside



3

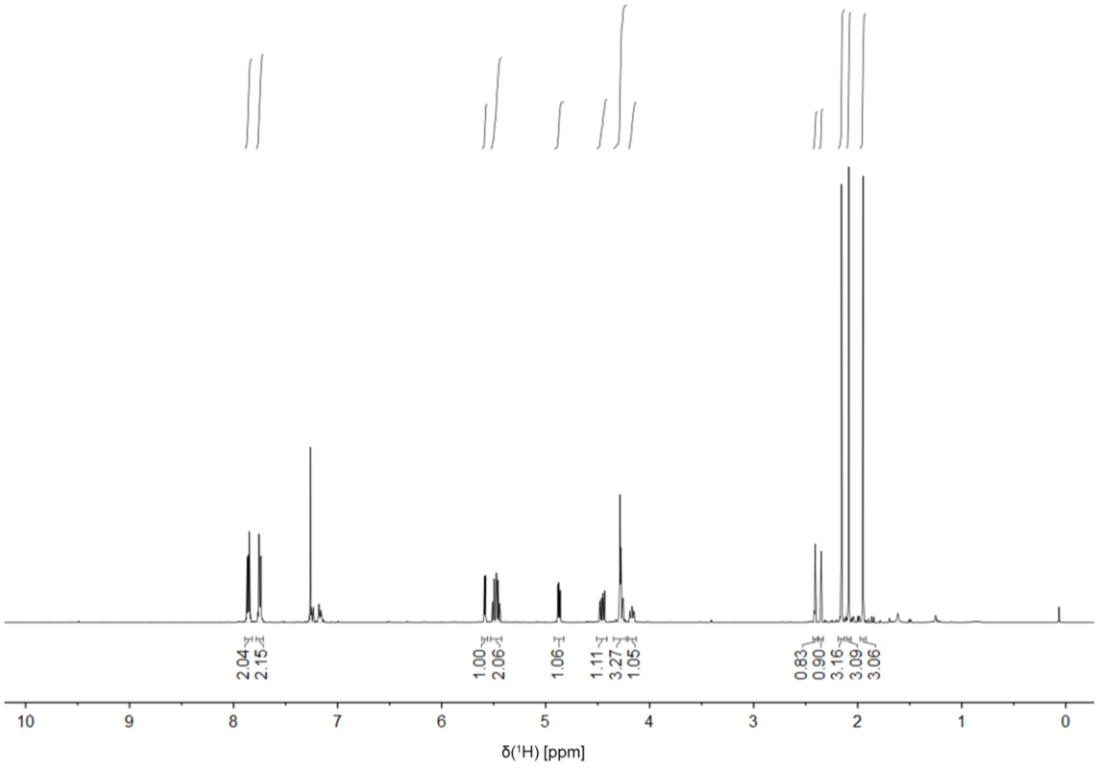
2 (1.40 g, 2.9 mmol) was dissolved in anhydrous DCM:ether (29 ml, 2:1). The mixture was stirred at 0°C and kept under argon. Propargyl alcohol (700 µl, 11.7 mmol) and $BF_3{\cdot}OEt_2$ (740 µl, 5.9 mmol) were added, the reaction was allowed to heat up to room temperature and stirred for 64 h. Progress of the reaction was monitored by analytical TLC (toluene:ethyl acetate (2:1)). The mixture was diluted in DCM (200 ml) and the organic phase was extracted with saturated $NaHCO_3$ and H_2O. Subsequently, the organic phase was dried with $MgSO_4$. Solvents were evaporated *in vacuo* and the residue was purified via column chromatography (toluene:ethyl acetate (8:1)) to yield **3** (752 mg, 1.57 mmol, 54%) as a light yellow resin. A stereoselectivity of 10 to 1 favoring the α-anomer was determined via ^{1}H-NMR experiments of the crude mixture. Starting material **2** that was not converted was recovered.

^{1}H NMR (400.0 MHz, $CDCl_3$): δ = 7.88 - 7.72 ppm, m, 4 H (aromatic H of Phth); δ = 5.58 ppm, d, 1H, J = 4.1 Hz (H1); δ = 5.48 ppm, dd, 1 H, J = 6.5, 8.0 Hz (H4); δ = 5.45 ppm, dd, 1 H, J = 4.9, 6.4 Hz (H3); δ = 4.87 ppm, dd, 1 H, J = 4.0 Hz, 4.9 Hz (H2); δ = 4.46 ppm, dd, 1 H, J = 6.1, 12.1 Hz (H6a); δ = 4.28 ppm, dd, 2 H, J = 0.5, 2.4 Hz (OC*H*$_2$CCH); δ = 4.27 ppm, dd, 1 H, J = 3.0, 12.1 Hz (H6b); δ = 4.17 ppm, ddd, 1 H, J = 3.0, 6.2, 8,0 Hz (H5); δ = 2.41, t, 1 H, J = 2.4 Hz (OCH$_2$CC*H*); δ = 2.16 ppm, s, 3 H (OCOC*H*$_3$); δ = 2.09 ppm, s, 3 H (OCOC*H*$_3$); δ = 1.95 ppm, s, 3 H (OCOC*H*$_3$).

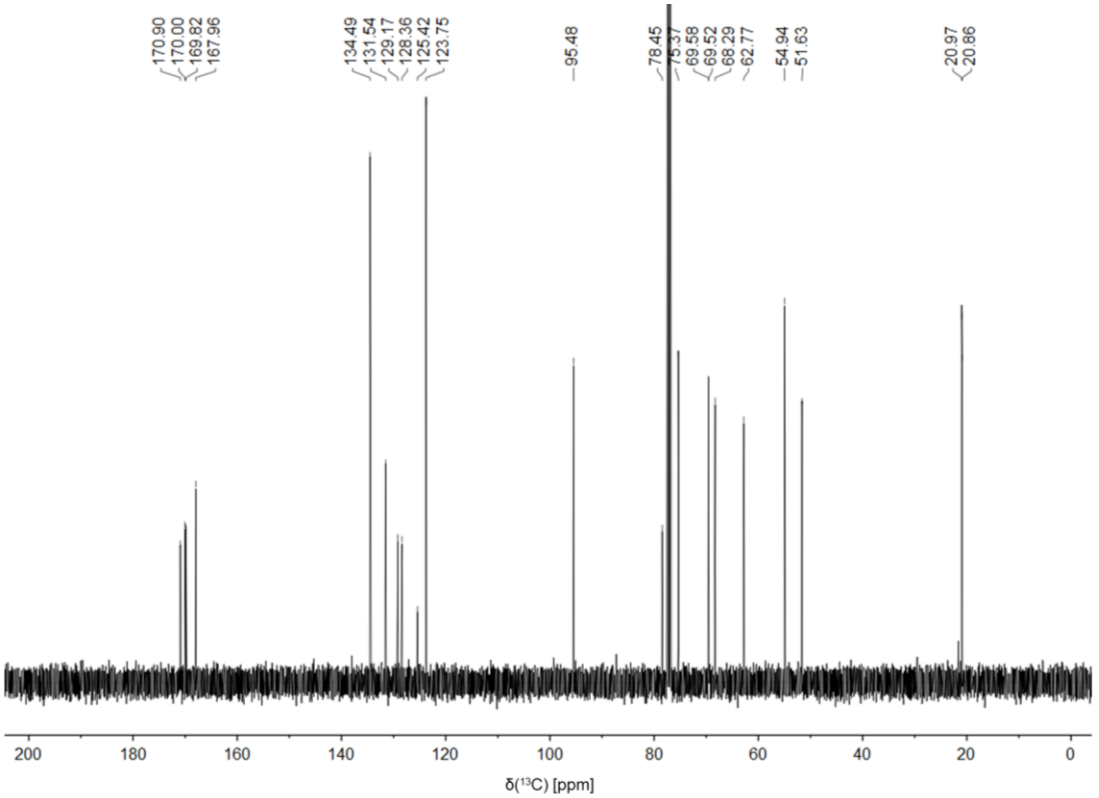
^{13}C NMR (100.6 MHz, CDCl3): δ = 170.9 ppm, 1 C (O*C*OCH$_3$); δ = 170.0 ppm, 1 C (O*C*OCH$_3$); δ = 169.8 ppm, 1 C (O*C*OCH$_3$); δ = 168.0 ppm, 2 C (carbonyl C of Phth); δ = 134.5 ppm, 2 C (aromatic C of Phth); δ = 131.5 ppm, 2 C (aromatic C of Phth); δ = 123.7 ppm, 2 C (aromatic C of Phth); δ = 95.5 ppm, 1 C (C1); δ = 78.5 ppm, 1 C (OCH$_2$*C*CH); δ = 75.4 ppm, 1 C (OCH$_2$C*C*H); δ = 69.6 ppm, 1 C (C5); δ = 69.5 ppm, 1 C (C3); δ = 68.3 ppm, 1 C (C4); δ = 62.8 ppm, 1 C (C6); δ = 54.9 ppm, 1 C (O*C*H$_2$CCH); δ = 51.6 ppm, 1 C (C2); δ = 21.0 ppm, 2 C (two times OCO*C*H$_3$); δ = 20.9 ppm, 1 C (OCO*C*H$_3$).

R_f = 0.47 with toluene:ethyl acetate (2:1).

ESI-MS for $C_{23}H_{23}NO_{10}$: $m{\cdot}z^{-1}(M+Na^+)_{calc}$ = 496.1; $m{\cdot}z^{-1}(M+Na^+)_{obs}$ = 496.0; $m{\cdot}z^{-1}(M+NH_4^+)_{calc}$ = 491.2; $m{\cdot}z^{-1}$ $(M+NH_4^+)_{obs}$ = 491.2.


2.04
2.15
1.00
2.06
1.06
1.11
3.27
1.05
0.83
0.90
3.16
3.09
3.06
δ(¹H) [ppm]

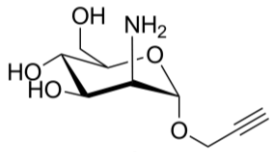
300


170.90
170.00
169.82
167.96
134.49
131.54
129.17
128.36
125.42
123.75
95.48
78.45
75.37
69.58
69.52
68.29
62.77
54.94
51.63
20.97
20.86
δ(¹³C) [ppm]

301

302

Propargyl-2-deoxy-2-amino-α-D-mannopyranoside



4

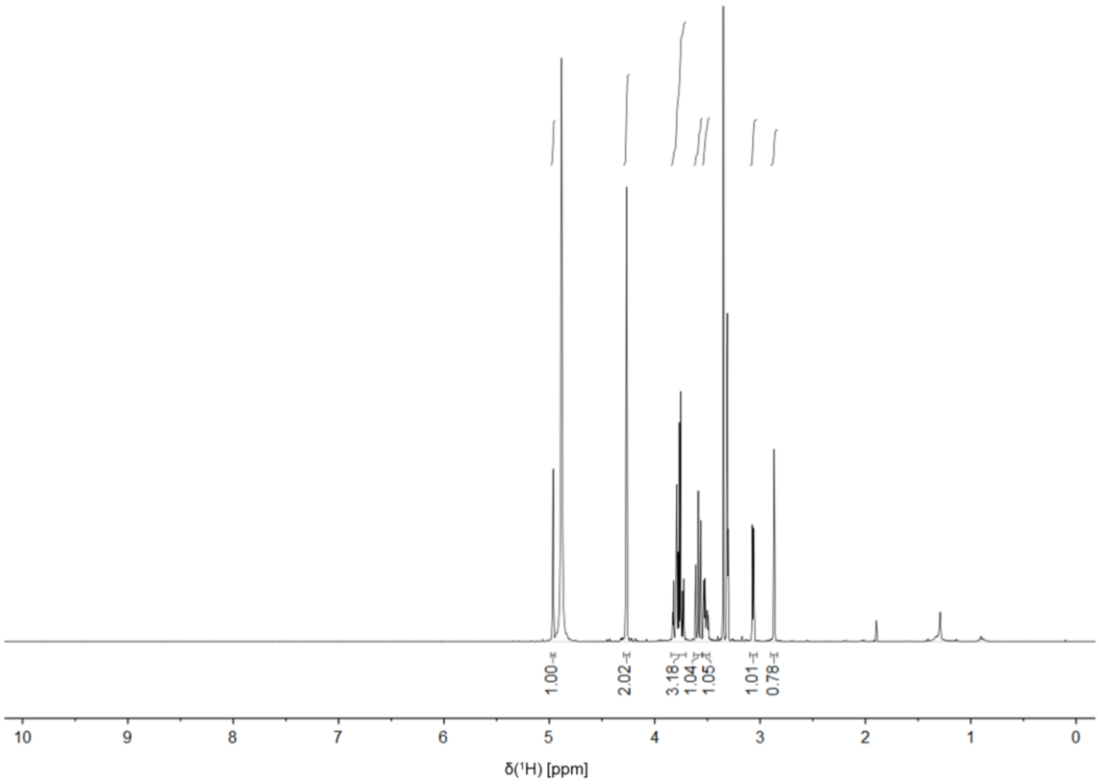
3 (2.70 g, 5.6 mmol) was dissolved in EtOH containing 33% methylamine (110 ml) at room temperature. The mixture was stirred overnight and progress of the reaction was monitored by analytical TLC (20% MeOH in DCM). Solvents were evaporated *in vacuo* and the residue was purified via column chromatography (gradient: hexane, hexane:DCM (1:1), DCM, 1% MeOH in DCM, 5% MeOH in DCM and elution with 20% MeOH in DCM). Silica gel particles were removed by filtration in MeOH with a cellulose acetate membrane at a pore size 0.2 µm to yield **4** (950 mg, 4.40 mmol, 78%) as a white solid.

^{1}H NMR (400.0 MHz, MeOD): δ = 4.95 ppm, d, 1 H, J = 1.3 Hz (H1); δ = 4.26 ppm, d, 2 H, J = 2.4 Hz (OC*H*$_2$CCH); δ = 3.79 ppm, dd, 1 H, J = 2.4, 11.9 Hz (H6a); δ = 3.76 ppm, dd, 1 H, J = 4.5, 9.3 Hz (H3); δ= 3.73 ppm, dd, 1 H, J = 4.7, 11.8 Hz (H6b); δ = 3.57 ppm, m, 1 H (H4); δ = 3.50 ppm, ddd, 1 H, J = 2.3, 4.8, 9.8 Hz (H5), δ = 3.05 ppm, dd, 1 H, J = 1.4, 4.3 Hz (H2); δ = 2.85 ppm, t, 1 H, J = 2.5 Hz (OCH$_2$CC*H*).

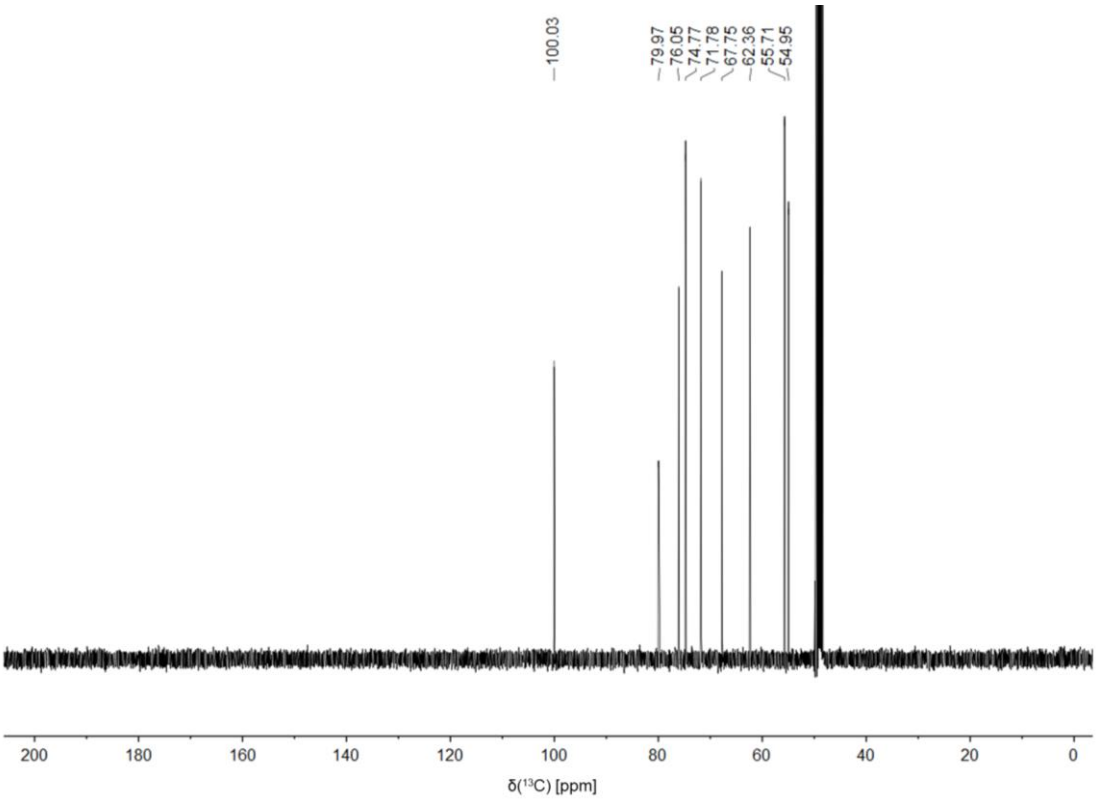
^{13}C NMR (100.6 MHz, MeOD): δ = 100.0 ppm, 1 C (C1); δ = 80.0 ppm, 1 C (OCH$_2$*C*CH); δ = 76.0 ppm, 1 C (OCH$_2$C*C*H); δ = 74.7 ppm, 1 C (C5); δ = 71.8 ppm, 1 C (C3); δ = 67.7 ppm, 1 C (C4); δ = 62.3 ppm, 1 C (C6); δ = 55.7 ppm, 1 C (C 2); δ = 54.9 ppm, 1 C (O*C*H$_2$CCH).

R_f = 0.22 with 20% MeOH in DCM.

HR ESI-MS for $C_9H_{15}NO_5$: $m \cdot z^{-1}(M+Na^+)_{calc}$ = 240.085, $m \cdot z^{-1}(M+Na^+)_{obs}$ = 240.085.


1.00
2.02
3.18
1.04
1.05
1.01
0.78
10
9
8
7
6
5
4
3
2
1
0
δ(¹H) [ppm]

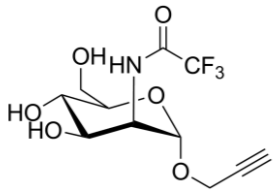
322


100.03
79.97
76.05
74.77
71.78
67.75
62.36
55.71
54.95
200
180
160
140
120
100
80
60
40
20
0
δ(¹³C) [ppm]

323

324

Propargyl-2-deoxy-2-2',2',2'-trifluoroacetamido-α-D-mannopyranoside



5.1

4 (255 mg, 1.2 mmol) was dissolved in DMF (2.4 ml) and ethyl 2,2,2-trifluoroacetate (155 µl, 1.3 mmol) was added at room temperature. Progress of the reaction was monitored by analytical TLC (10% MeOH in DCM). After 6 hours solvents were evaporated *in vacuo* and the residue was purified via column chromatography (gradient: DCM and elution with ml 5% MeOH in DCM). Silica gel particles were removed by filtration in MeOH with a cellulose acetate membrane at a pore size 0.2 µm to yield **5.1** (248 mg, 790 µmol, 67%) as a white solid.

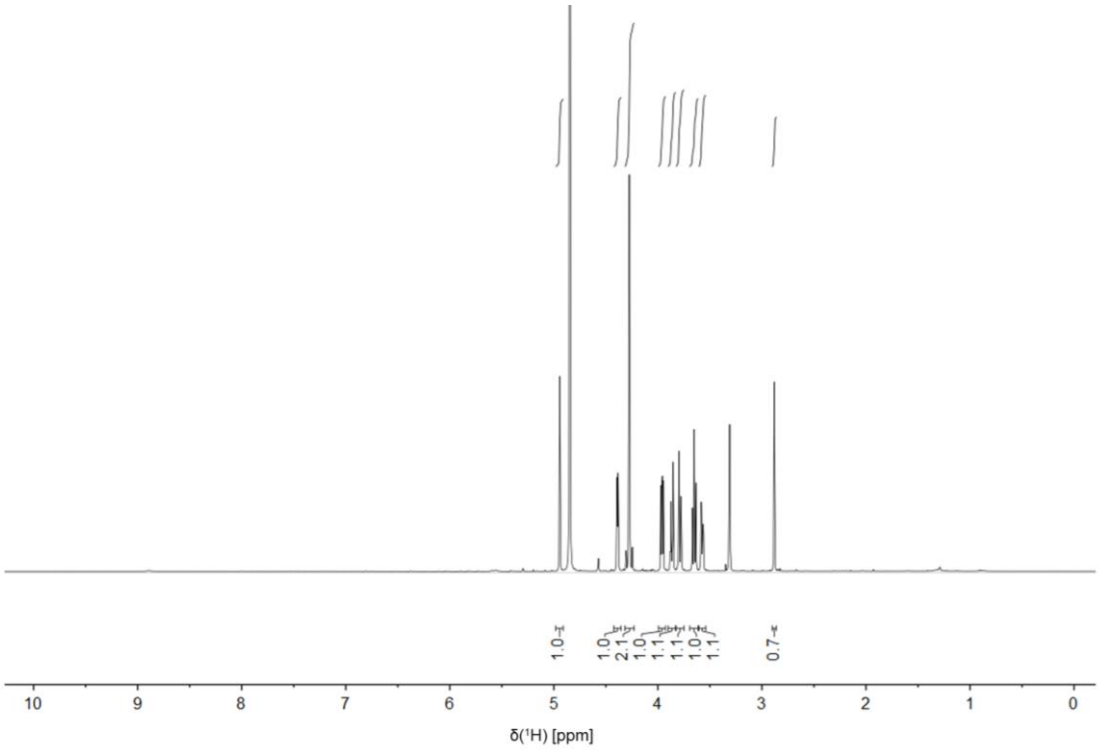
^{1}H NMR (600.0 MHz, MeOD)**:** δ = 4.94 ppm, d, 1 H, J = 0.8 Hz (H1); δ = 4.39 ppm, dd, 1 H, J = 0.8, 4.9 Hz (H2); δ = 4.27 ppm, m, 2 H (OC*H*$_2$CCH); δ = 3.96 ppm, dd, 1 H, J = 4.8, 9.7 Hz (H3); δ = 3.87 ppm, dd, 2 H, J = 4.0, 11.7 Hz (H6a); δ = 3.79 ppm, dd, 2 H, J = 2.4, 11.7 Hz (H6b); δ = 3.65 ppm, t, 1 H, J = 9.8 Hz (H4); δ = 3.57 ppm, ddd, 1 H, J = 2.2, 3.8, 9.9 Hz (H5); δ = 2.88 ppm, t, 1 H, J = 2.5 Hz (OCH$_2$CC*H*).

^{13}C NMR (100.6 MHz, MeOD): δ = 159.6 ppm, q, 1C, J = 37.6 Hz (NH*C*OCF$_3$); δ = 117.3 ppm, q, 1C, J = 286.6 Hz (NHCO*C*F$_3$); δ = 98.5 ppm, 1 C (C1); δ = 79.6 ppm, 1 C (OCH$_2$*C*CH); δ = 76.4 ppm, 1 C (OCH$_2$C*C*H); δ = 74.5 ppm, 1 C (C5); δ = 70.5 ppm, 1 C (C3); δ = 67.5 ppm, 1 C (C4); δ = 61.5 ppm, 1 C (C6); δ = 55.3 ppm, 1 C (O*C*H$_2$CCH); δ = 54.8 ppm, 1 C (C2).

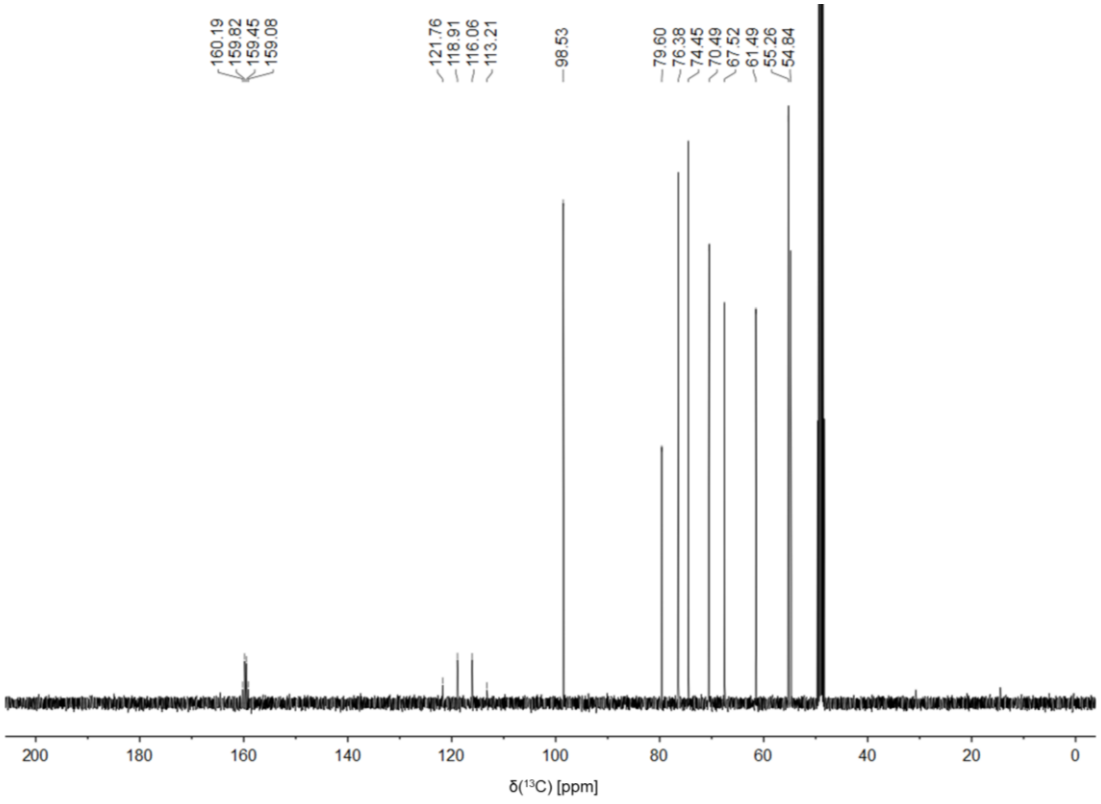
^{19}F NMR (376.0 MHz, D$_2$O): δ = -75.8 ppm, s, 3 F (C*F*$_3$).

R_f = 0.39 with 10% in MeOH in DCM.

HR ESI-MS for $C_{11}H_{14}F_3NO_6$: $m \cdot z^{-1}(M+Na^+)_{calc}$ = 336.067, $m \cdot z^{-1}(M+Na^+)_{obs}$ = 336.070.


1.0
1.0
2.1
1.0
1.1
1.1
1.0
1.1
0.7
10
9
8
7
6
5
4
3
2
1
0
δ(¹H) [ppm]

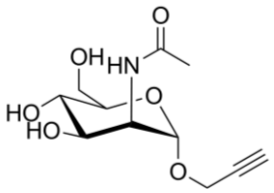
345


160.19
159.82
159.45
159.08
121.76
118.91
116.06
113.21
98.53
79.60
76.38
74.45
70.49
67.52
61.49
55.26
54.84
200
180
160
140
120
100
80
60
40
20
0
δ(¹³C) [ppm]

346

347

Propargyl-2-deoxy-2-acetamido-α-D-mannopyranoside



5.2

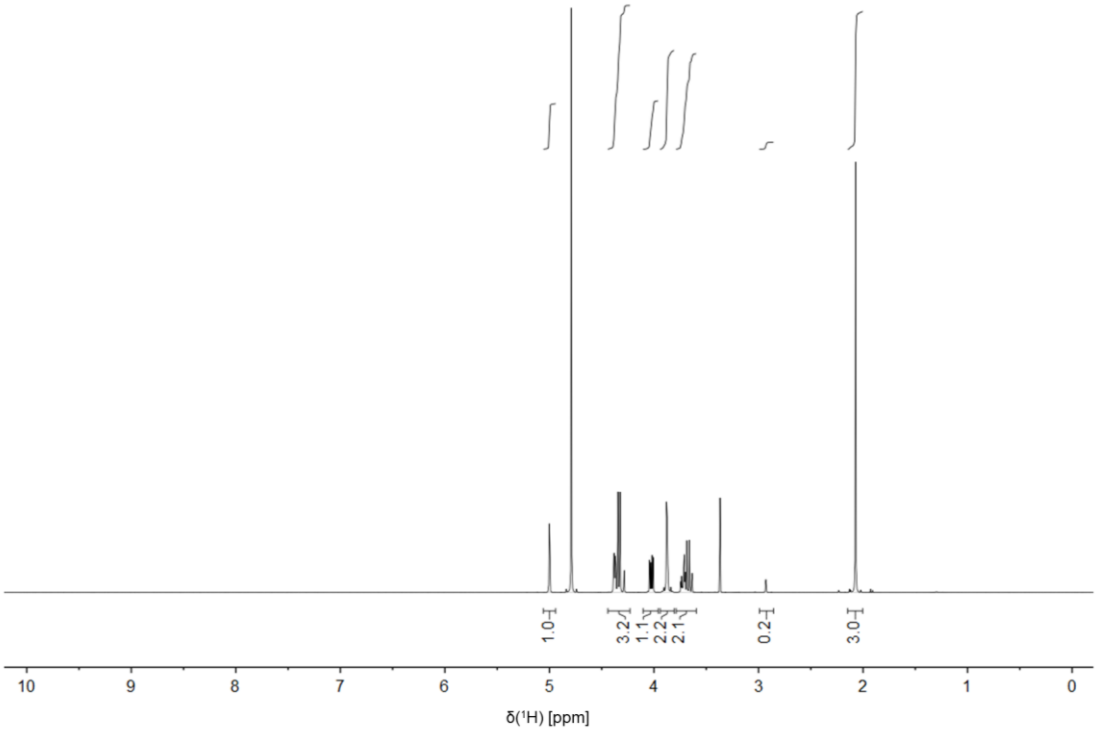
4 (59 mg, 270 µmol) was dissolved in MeOH (5 ml) and acetic anhydride (194 mg, 1.9 mmol) was added. The mixture was stirred at room temperature overnight. Progress of the reaction was monitored by analytical TLC (5% MeOH in DCM). Solvents were evaporated *in vacuo* and the residue was purified via column chromatography (gradient: hexane, hexane:DCM (1:1), DCM, 5% MeOH in DCM and elution with 20% MeOH in DCM). Silica gel particles were removed by filtration in MeOH with a cellulose acetate membrane at a pore size 0.2 µm to yield **5.2** (57 mg, 220 µmol, 81%) as a white solid.

^{1}H NMR (400.0 MHz, D_2O): δ = 5.00 ppm, d, 1 H, J = 1.1 Hz (H1); δ = 4.37 ppm, dd, 1 H, J = 1.1, 4.8 Hz (H2); δ = 4.34 ppm, m, 2 H (OC*H*$_2$CCH); δ = 4.02 ppm, dd, 1 H, J = 4.8, 9.4 Hz (H3); δ = 3.88 ppm, m, 2 H (H6a/b); δ = 3.71 ppm, dt, 1 H, J = 3.5, 10.0 Hz (H 5); δ = 3.66 ppm, m, 1 H (H4); δ = 2.93 ppm, t, 1 H, J = 2.5 Hz (OCH$_2$CC*H*); δ = 2.07 ppm, s, 3 H (NHCOC*H*$_3$).

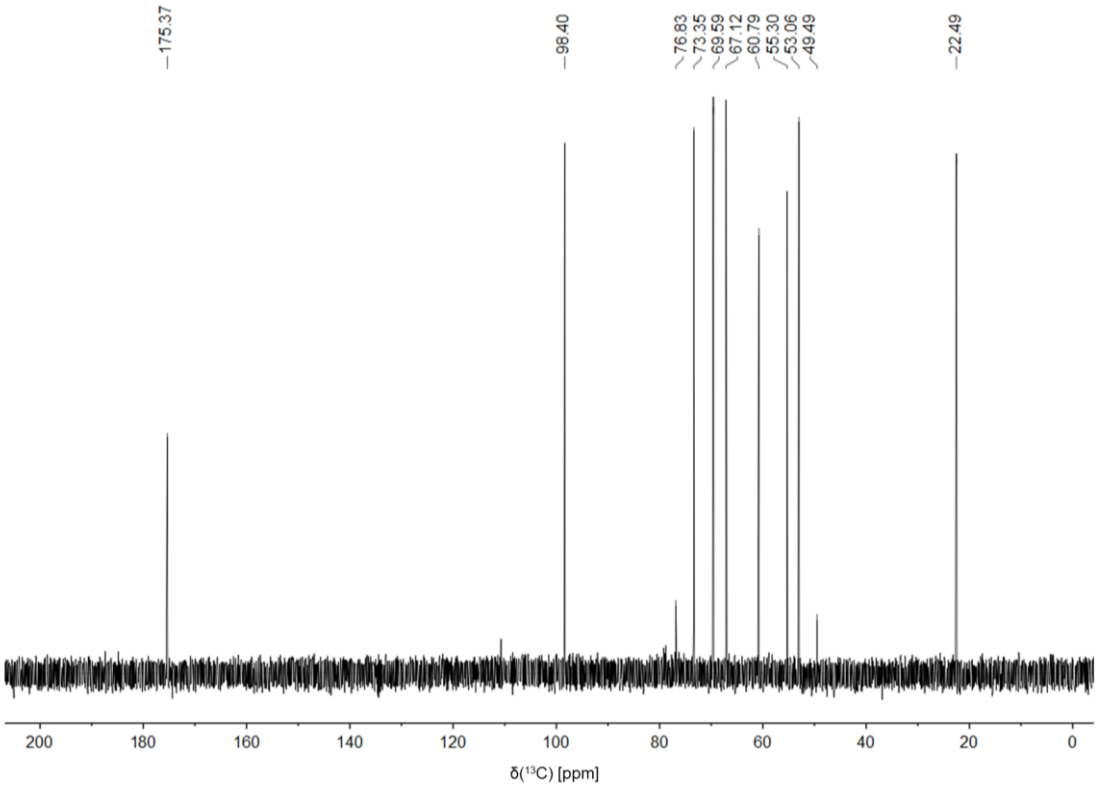
^{13}C NMR (100.6 MHz, D_2O): δ = 175.4 ppm, 1 C (NH*C*OCH$_3$); δ = 98.4 ppm, 1 C (C1); δ = 78.8 ppm, 1 C (OCH$_2$*C*CH); δ = 76.2 ppm, 1 C (OCH$_2$C*C*H); δ = 73.3 ppm, 1 C (C5); δ = 69.6 ppm, 1 C (C3); δ = 67.1 ppm, 1 C (C4); δ = 60.8 ppm, 1 C (C6); δ = 55.3 ppm, 1 C (O*C*H$_2$CCH); δ = 53.0 ppm, 1 C (C2); δ = 22.5 ppm, 1 C (NHCO*C*H$_3$).

R_f = 0.27 with 10% MeOH in DCM.

HR ESI-MS for $C_{11}H_{17}NO_6$: $m{\cdot}z^{-1}(M+Na^+)_{calc}$ = 282.095; $m{\cdot}z^{-1}(M+Na^+)_{obs}$ = 282.095.


1.0
3.2
1.1
2.2
2.1
0.2
3.0
10
9
8
7
6
5
4
3
2
1
0
δ(¹H) [ppm]

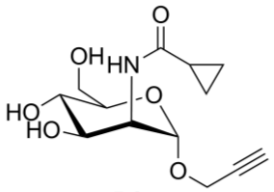
367


175.37
98.40
76.83
73.35
69.59
67.12
60.79
55.30
53.06
49.49
22.49
200
180
160
140
120
100
80
60
40
20
0
δ(¹³C) [ppm]

368

369

Propargyl-2-deoxy-2-cyclopropanecarboxmido-α-D-mannopyranoside



5.3

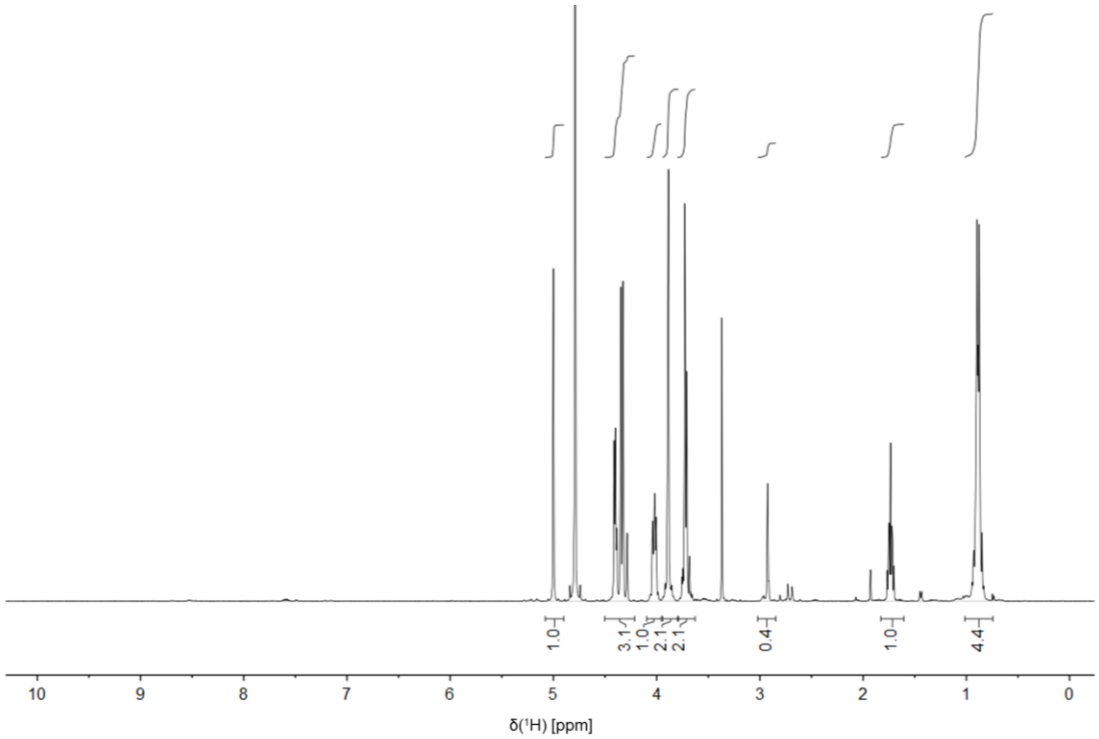
4 (48 mg, 220 µmol) was dissolved in pyridine (5 ml) and cyclopropanecarbonyl chloride (160 µl, 1.8 mmol, Acros Organics) was added at 0°C. The mixture was allowed to heat up to room temperature and progress of the reaction was monitored by analytical TLC (3% MeOH in DCM). After 7 h EtOH containing 33% methylamine (10 ml) was added and the mixture was stirred overnight at room temperature. Solvents were evaporated *in vacuo* and the residue was purified via column chromatography (gradient: hexane, hexane:DCM (1:1), DCM and elution with ml 1% MeOH in DCM) to yield **5.3** (27 mg, 95 µmol, 43%) as a yellow solid.

^{1}H NMR (400.0 MHz, D_2O): δ = 5.00 ppm, d, 1 H, J = 1.3 Hz (H1); δ = 4.40 ppm, dd, 1 H, J = 1.4, 4.8 Hz (H2); δ = 3.34 ppm, m, 2 H (OC*H2*CCH); δ = 4.02 ppm, m, 1 H (H3); δ = 3.89 ppm, m, 2 H (H6a/b); δ = 3.72, m, 2 H (H4, H5); δ = 2.93 ppm, t, J = 2.3 Hz (OCH_2CC*H*); δ = 1.73 ppm, m, 1 H ($NHCOC$*H*C_2H_4); δ = 0.89 ppm, m, 4 H ($NHCOCHC_2$*H*$_4$).

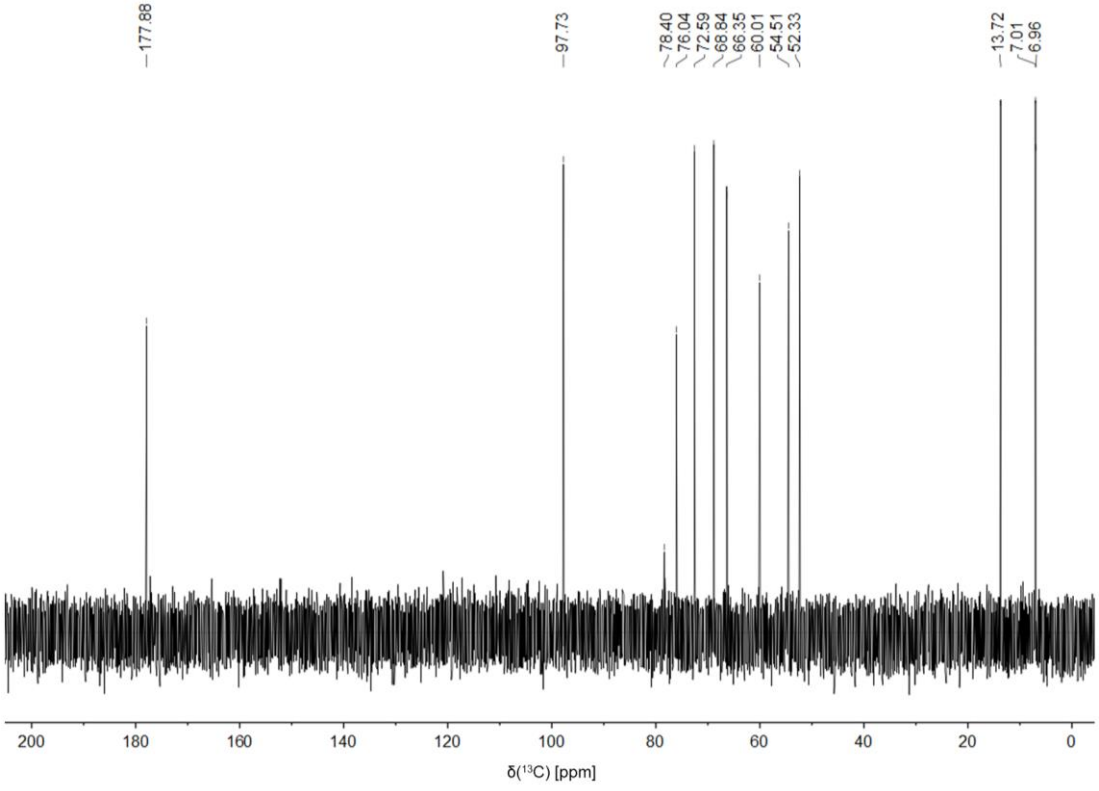
^{13}C NMR (100.6 MHz, D_2O): δ = 178.4 ppm, 1C ($NHCOCHC_2H_4$); δ = 98.3 ppm, 1 C (C1); δ = 79.1 ppm, 1 C (OCH_2CCH); δ = 76.7 ppm, 1 C (OCH_2CCH); δ = 73.2 ppm, 1 C (C5); δ = 69.5, 1 C (C3); δ = 67.0 ppm, 1 C (C4); δ = 60.7 ppm, 1 C (C6); δ = 55.2, 1 C (OCH_2CCH); δ = 53.0 ppm, 1 C (C2); δ = 14.4 ppm, 1 C ($NHCOCHC_2H_4$); δ = 7.7 ppm, 1 C ($NHCOCHC_2H_4$); δ = 7.6 ppm, 1 C ($NHCOCHC_2H_4$).

R_f = 0.51 with 3% MeOH in DCM

HR ESI-MS for $C_{13}H_{19}NO_6$: $m{\cdot}z^{-1}(M+Na^+)_{calc}$ = 308.111; $m{\cdot}z^{-1}(M+Na^+)_{obs}$ = 308.111.


1.0
3.1
1.0
2.1
2.1
0.4
1.0
4.4
10
9
8
7
6
5
4
3
2
1
0
δ(¹H) [ppm]

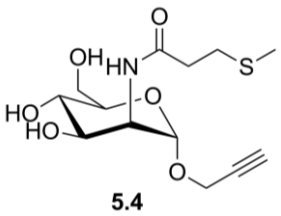
390


177.88
97.73
78.40
76.04
72.59
68.84
66.35
60.01
54.51
52.33
13.72
7.01
6.96
200
180
160
140
120
100
80
60
40
20
0
δ(¹³C) [ppm]

391

392

Propargyl-2-deoxy-2-3'-(methylthio)propanamido-α-D-mannopyranoside



5.4

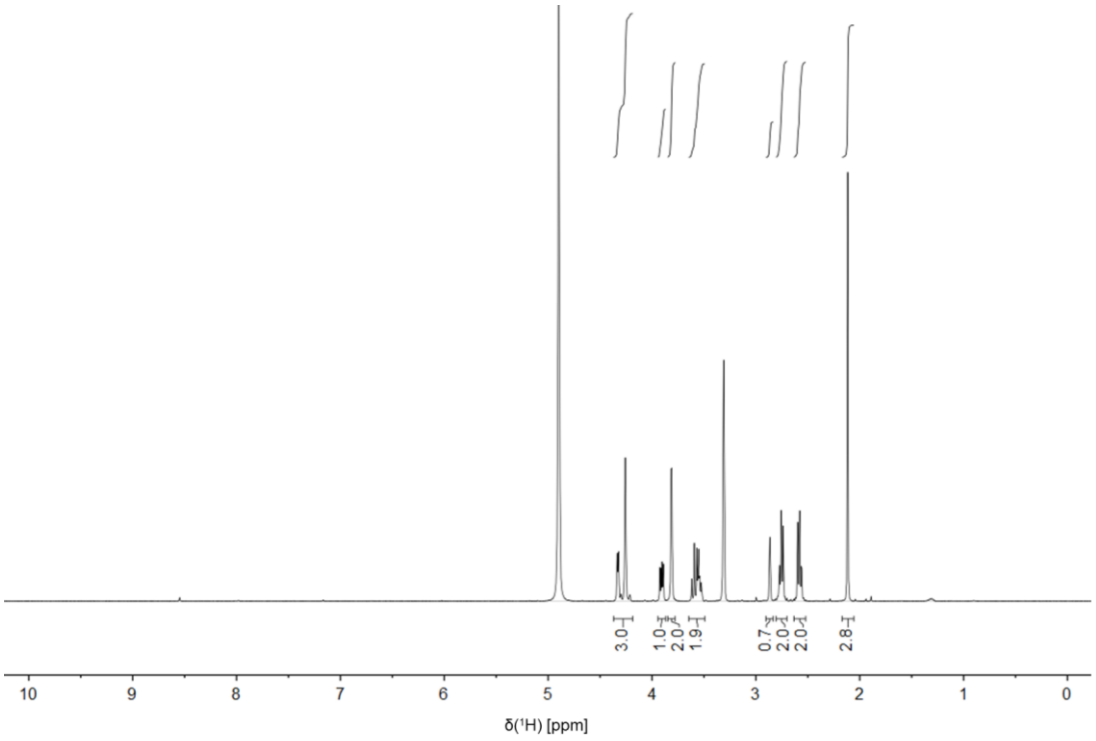
4 (3.03 mg, 150 µmol) was dissolved in pyridine (760 µl) and 3-(methylthio)propanoyl chloride (21 µl, 170 µmol, TCI) was added at 0°C. The reaction mixture was allowed to heat up to room temperature and after 4 h more 3-(methylthio)propanoyl chloride (21 µl, 170 µmol, TCI) was added. Progress of the reaction was monitored by analytical TLC (10% MeOH in DCM) and after 7 h EtOH containing 33% methylamine (5 ml) was added at room temperature. The mixture was stirred overnight, solvents were evaporated *in vacuo* and the residue was purified via column chromatography (gradient: hexane, DCM, 1% MeOH in DCM, 5% MeOH in DCM and elution with 10% MeOH in DCM). Residual impurities were removed via reversed-phase column chromatography (gradient: H_2O, 1% MeOH in H_2O and elution with 5% MeOH in H_2O) to yield **5.4** (4.8 mg, 15 µmol, 10%) as a white solid.

^{1}H NMR (400.0 MHz, MeOD): δ = 4.90 ppm, s, 1 H (H1); δ = 4.33 ppm, d, 1 H, J = 4.6 Hz (H2); δ = 4.26 ppm, m, 2 H (OC*H2*CCH); δ = 3.91 ppm, dd, J = 4.7, 9.1 Hz, 1 H (H3); δ = 3.81 ppm, d, 2 H, J = 3.2 Hz (H6a/b); δ = 3.59, m, 1 H (H4); δ = 3.54, dt, 1 H, J = 3.1, 10.0 Hz (H5); δ = 2.87 ppm, t, J = 2.2 Hz (OCH_2CC*H*); δ = 2.75 ppm, m, 2 H ($NHCOCH_2C$*H*$_2SCH_3$); δ = 2.58 ppm, m, 2 H ($NHCOC$*H*$_2CH_2SCH_3$); δ = 2.12 ppm, s, 3 H ($NHCOCH_2CH_2SC$*H*$_3$).

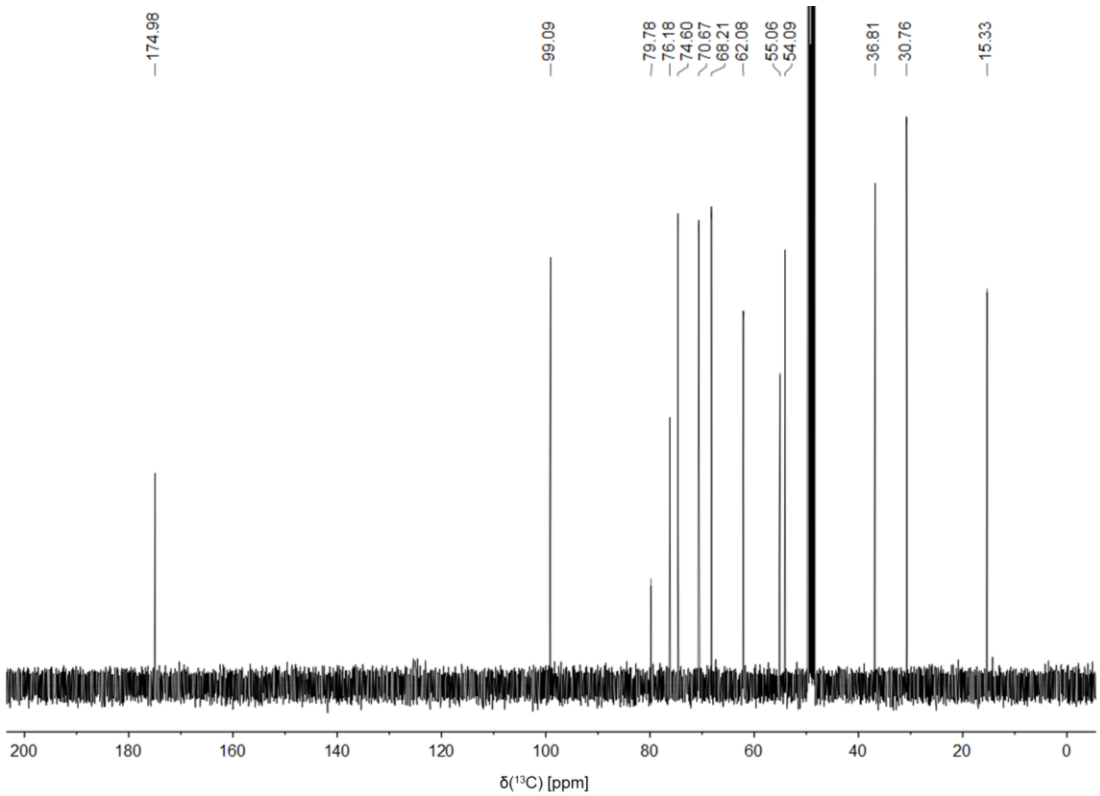
^{13}C NMR (100.6 MHz, MeOD): δ = 175.0 ppm, 1C ($NHCOCH_2CH_2SCH_3$); δ = 99.1 ppm, 1 C (C1); δ = 79.8 ppm, 1 C (OCH_2CCH); δ = 76.2 ppm, 1 C (OCH_2CCH); δ = 74.6 ppm, 1 C (C5); δ = 70.7, 1 C (C3); δ = 68.2 ppm, 1 C (C4); δ = 62.1 ppm, 1 C (C6); δ = 55.1, 1 C (OCH_2CCH); δ = 54.1 ppm, 1 C (C2); δ = 36.8 ppm, 1 C ($NHCOCH_2CH_2SCH_3$); δ = 30.8 ppm, 1 C ($NHCOCH_2CH_2SCH_3$); δ = 15.3 ppm, 1 C ($NHCOCH_2CH_2SCH_3$).

R_f = 0.18 with 10% MeOH in DCM.

HR ESI-MS for $C_{13}H_{21}NO_6S$: $m{\cdot}z^{-1}(M+Na^+)_{calc}$ = 342.099; $m{\cdot}z^{-1}(M+Na^+)_{obs}$ = 342.103.


3.0
1.0
2.0
1.9
0.7
2.0
2.0
2.8
10
9
8
7
6
5
4
3
2
1
0
δ(¹H) [ppm]

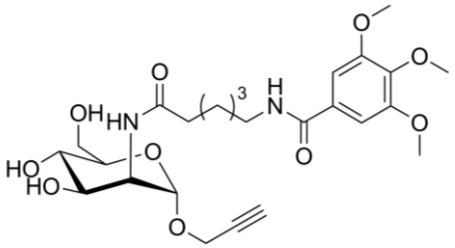
417


174.98
99.09
79.78
76.18
74.60
70.67
68.21
62.08
55.06
54.09
36.81
30.76
15.33
200
180
160
140
120
100
80
60
40
20
0
δ(¹³C) [ppm]

418

419

Propargyl-2-deoxy-2-*N*-(6'-amino-6'-oxohexyl)-3',4',5'-trimethoxybenzamide-α-D-mannopyranoside



5.5

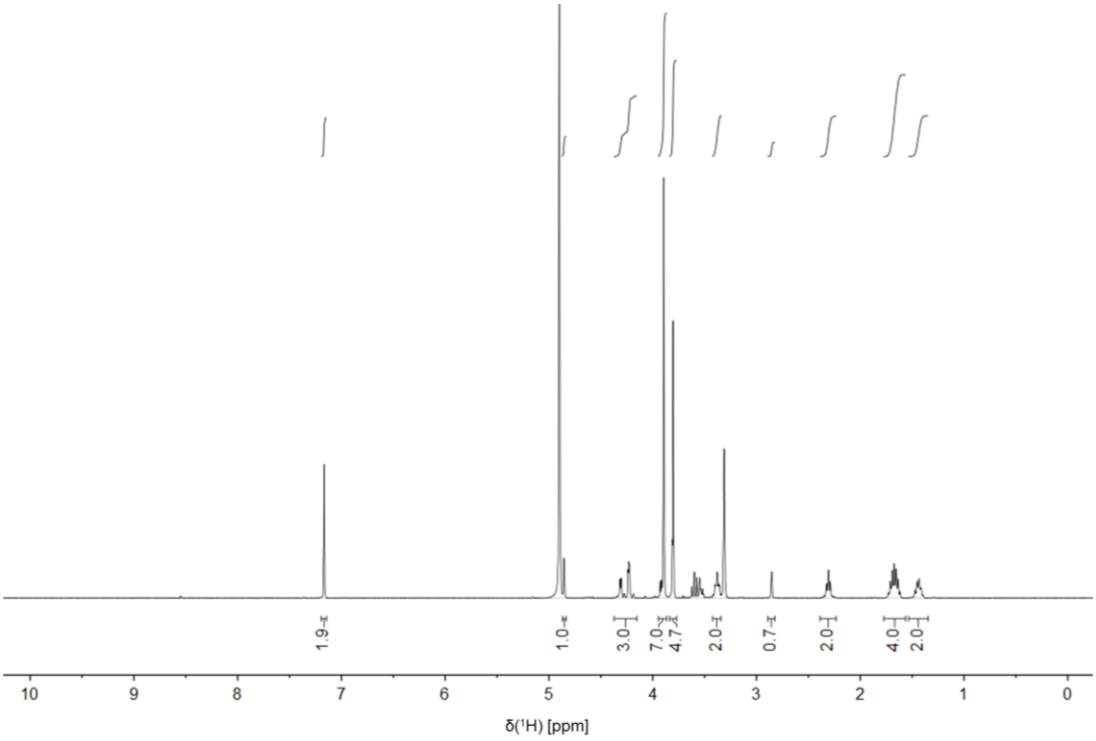
6-(3,4,5-Trimethoxybenzamido)hexanoic acid (90 mg, 0.28 mmol, Vitas-M Laboratory) was dissolved in DMF (700 µl). Subsequently, PyBOP (150 mg, 0.28 mmol) and DIPEA (100 µl, 0.55 mmol) were added and the mixture was stirred for 10 min at room temperature. **4** (30 mg, 0.14 mmol) was added and the mixture was stirred overnight at room temperature. Progress of the reaction was monitored by analytical TLC (10% MeOH in DCM). The reaction was quenched with MeOH (1 ml) and after addition of 1 M NaOH (830 µl, 0.83 mmol) solvents were removed *in vacuo*. The residue was purified via reversed-phase column chromatography (gradient: H_2O, 10 % MeOH in H_2O, 20 % MeOH in H_2O, 30 % MeOH in H_2O and elution with 40 % MeOH in H_2O) to yield **5.5** (51 mg, 97 µmol, 70 % yield) as a white solid.

^{1}H NMR (400.0 MHz, MeOD): δ = 7.17 ppm, s, 2 H (aromatic H of 3',4',5'-trimethoxybenzamide); δ = 4.85 ppm, s, 1 H (H1); δ = 4.31 ppm, d, 1 H, J = 5.1 Hz (H2); δ = 4.23 ppm, m, 2 H (OC*H*$_2$CCH); δ = 3.91 ppm, m, 1 H (H3); δ = 3.89 ppm, s, 6 H (two times CH_3 of 3',4',5'-trimethoxybenzamide); δ = 3.81 ppm, m, 2 H, (H6a/b); δ = 3.81 ppm, s, 3 H (CH_3 of 3',4',5'-trimethoxybenzamide); δ = 3.60 ppm, m, 1 H (H4); δ = 3.53 ppm, dt, 1 H, J = 3.2, 10.1 Hz (H5); δ = 3.38 ppm, m, 2 H (NHCOCH$_2$CH$_2$CH$_2$CH$_2$C*H*$_2$NHCO); δ = 2.85 ppm, t, J = 2.4 Hz (OCH$_2$CC*H*); δ = 2.31 ppm, m, 2 H (NHCOC*H*$_2$CH$_2$CH$_2$CH$_2$CH$_2$NHCO); δ = 1.67 ppm, m, 4 H (NHCOCH$_2$C*H*$_2$CH$_2$C*H*$_2$CH$_2$NHCO); δ = 1.43 ppm, m, 2 H (NHCOCH$_2$CH$_2$C*H*$_2$CH$_2$CH$_2$NHCO).

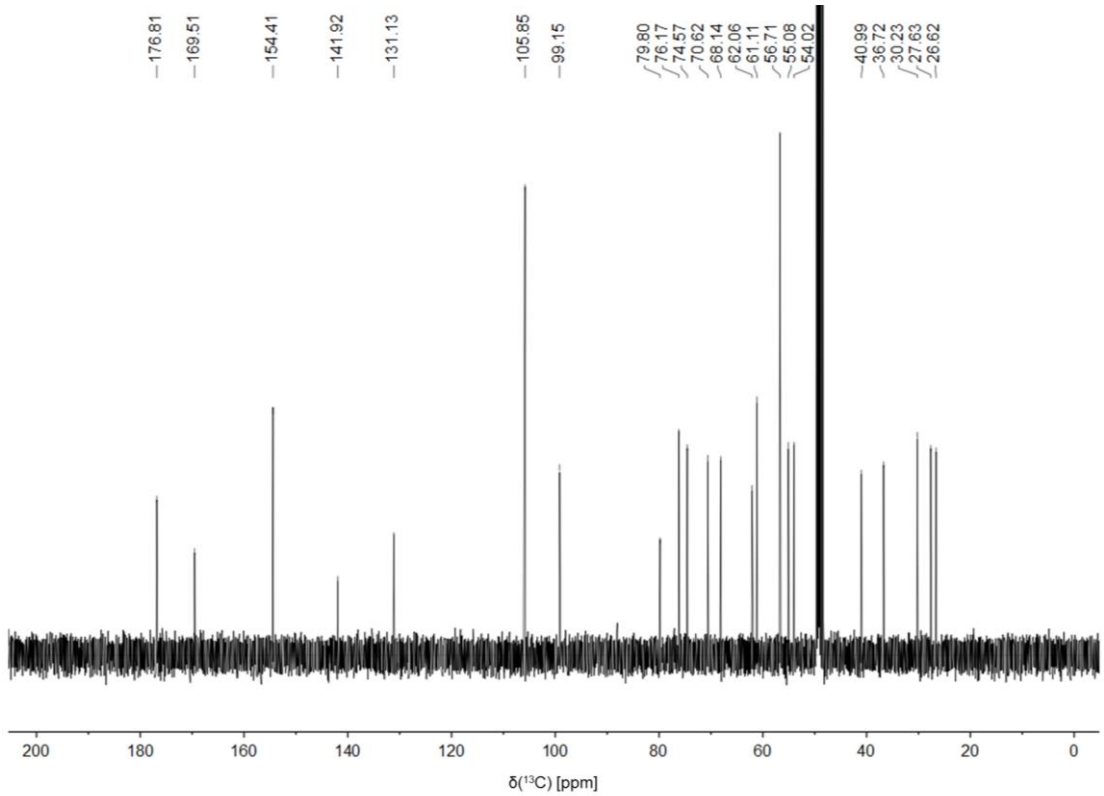
441 ^{13}C NMR (100.6 MHz, MeOD): δ = 175.0 ppm, 1C (NH*C*OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$NHCO); δ = 169.5
442 ppm, 1C (NHCOCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$NH*C*O); δ = 154.4 ppm, 2C (C3' and C5' of 3',4',5'-
443 trimethoxybenzamide); δ = 141.9 ppm, 1C (C4' of 3',4',5'-trimethoxybenzamide); δ = 131.1 ppm, 1C
444 (C1' of 3',4',5'-trimethoxybenzamide); δ = 105.9 ppm, 2C (C2' and C6' of 3',4',5'-
445 trimethoxybenzamide); δ = 99.2 ppm, 1 C (C1); δ = 79.80 ppm, 1 C (OCH$_2$*C*CH); δ = 76.2 ppm, 1 C
446 (OCH$_2$C*C*H); δ = 74.6 ppm, 1 C (C5); δ = 70.69, 1 C (C3); δ = 68.2 ppm, 1 C (C4); δ = 62.1 ppm, 1 C
447 (C6); δ = 61.1 ppm, 1 C (CH$_3$ of 3',4',5'-trimethoxybenzamide); δ = 56.7 ppm, 2 C (two times CH$_3$ of
448 3',4',5'-trimethoxybenzamide); δ = 55.1, 1 C (O*C*H$_2$CCH); δ = 54.0 ppm, 1 C (C2); δ = 41.0 ppm, 1 C
449 (NHCOCH$_2$CH$_2$CH$_2$CH$_2$*C*H$_2$NHCO); δ = 36.7 ppm, 1 C (NHCO*C*H$_2$CH$_2$CH$_2$CH$_2$CH$_2$NHCO); δ =
450 30.2 ppm, 1 C (NHCOCH$_2$CH$_2$CH$_2$*C*H$_2$CH$_2$NHCO); δ = 27.6 ppm, 1 C
451 (NHCOCH$_2$CH$_2$*C*H$_2$CH$_2$CH$_2$NHCO); δ = 26.6 ppm, 1 C (NHCOCH$_2$*C*H$_2$CH$_2$CH$_2$CH$_2$NHCO).

452 R_f = 0.23 with 10% MeOH in DCM.

453 HR ESI-MS for $C_{25}H_{36}N_2O_{10}$: m·z^{-1}(M+Na$^+$)$_{calc}$ = 547.227; m·z^{-1}(M+Na$^+$)$_{obs}$ = 547.234.



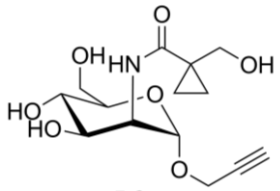
454


176.81
169.51
154.41
141.92
131.13
105.85
99.15
79.80
76.17
74.57
70.62
68.14
62.06
61.11
56.71
55.08
54.02
40.99
36.72
30.23
27.63
26.62
200
180
160
140
120
100
80
60
40
20
0
δ(13C) [ppm]

455

456

Propargyl-2-deoxy-2-1'-hydroxycyclopropanecarboxamide-α-D-mannopyranoside



5.6

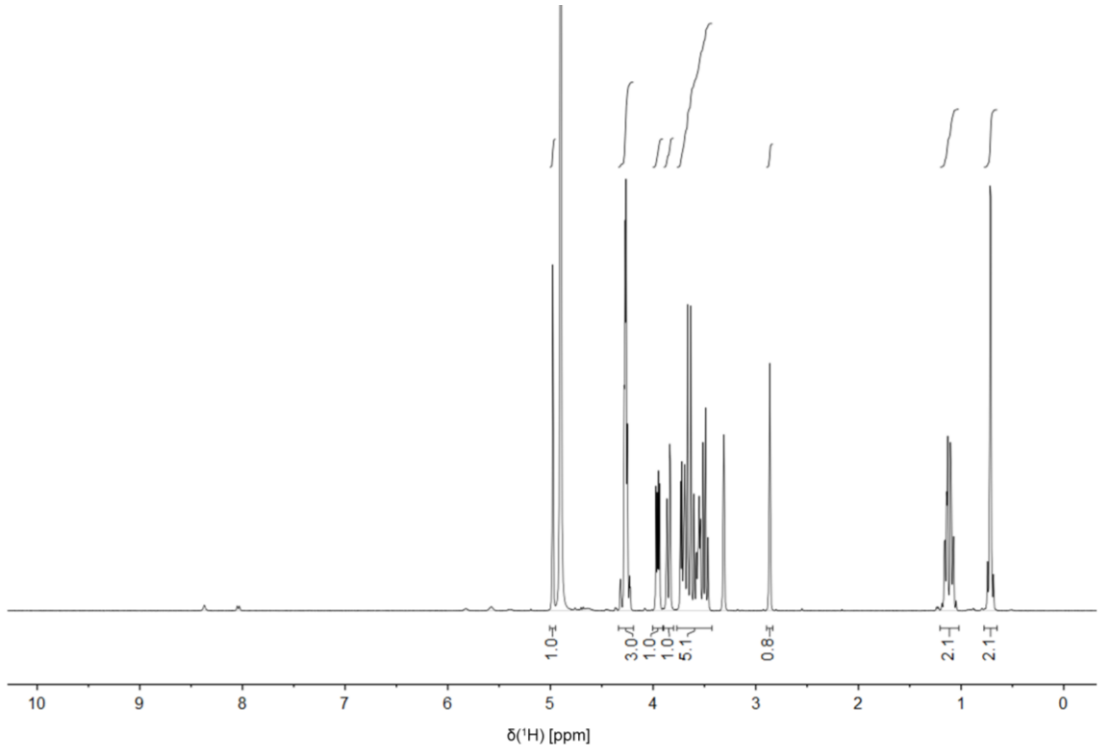
1-hydroxycyclopropanecarboxylic acid (37 mg, 0.32 mmol, ChemBridge) was dissolved in DMF (800 µl). Subsequently, PyBOP (170 mg, 0.32 mmol) and DIPEA (120 µl, 0.65 mmol) were added and the mixture was stirred for 10 min at room temperature. **4** (35 mg, 0.16 mmol) was added, the mixture was stirred overnight at room temperature. Progress of the reaction was monitored by analytical TLC (15% MeOH in DCM). The reaction was quenched with MeOH (1 ml) and after addition of 1 M NaOH (1000 µl, 1.0 mmol) solvents were removed *in vacuo*. The residue was purified via preparative HPLC (gradient: H_2O for 10 min, from 0 to 20% acetonitrile in H_2O in 30 min, from 20 to 50% acetonitrile in H_2O in 10 min, from 50 to 100% acetonitrile in H_2O in 5 min and acetonitrile for 5 min at 3.2 ml·min^{-1}) to yield **5.6** (16 mg, 49 µmol, 31%) as a white solid.

^{1}H NMR (400.0 MHz, MeOD): δ = 4.98 ppm, s, 1 H (H1); δ = 4.25 ppm, m, 2 H (OC*H2*CCH); δ = 4.23 ppm, m, 1 H (H2); δ = 3.95 ppm, dd, 1 H, J = 4.7, 9.3 Hz (H3); δ = 3.85 ppm, dd, 1 H, J = 1.9, 11.9 Hz (H6a); δ = 3.71 ppm, m, 1 H (H6b); δ = 3.64 ppm, m, 2 H (NHCOC(CH_2CH_2)C*H*$_2$OH); δ = 3.55, m, 1 H (H5); δ = 3.59, m, 1 H (H4); δ = 2.87 ppm, m, 1 H (OCH_2CC*H*); δ = 1.12 ppm, m, 2 H (NHCOC(C*H*$_2$CH$_2$)CH_2OH); δ = 0.71 ppm, m, 2 H (NHCOC(CH_2C*H*$_2$)CH_2OH).

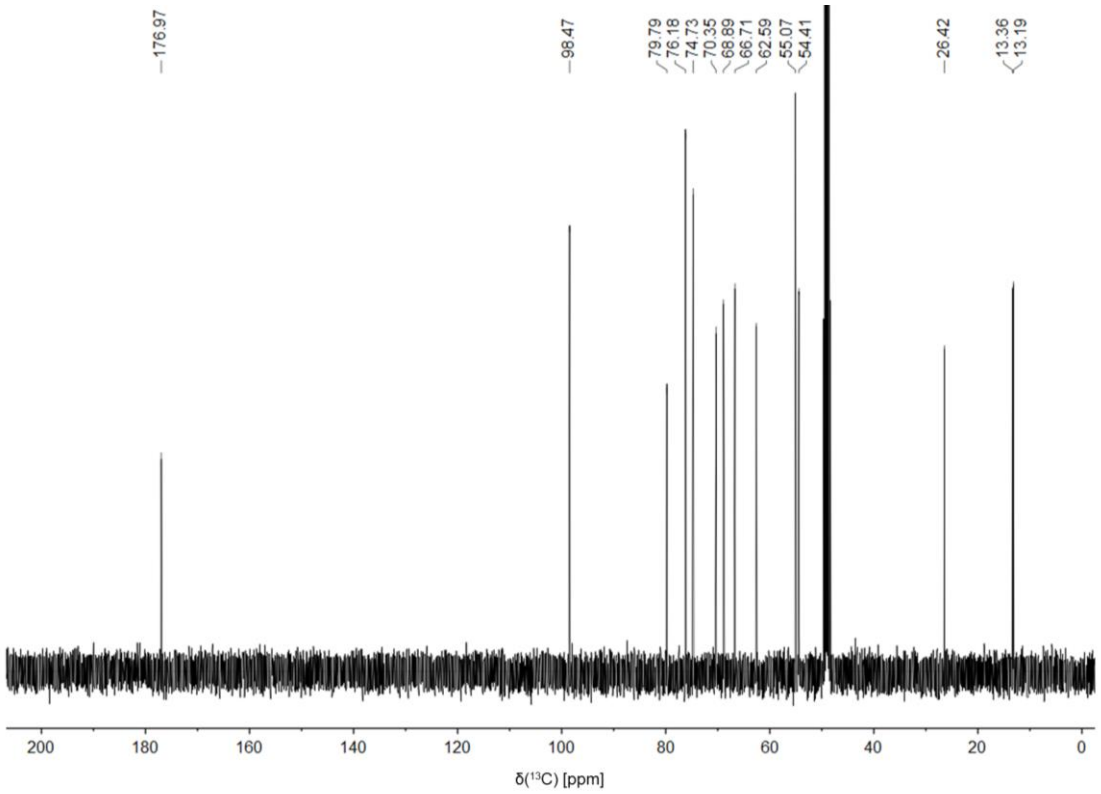
^{13}C NMR (100.6 MHz, MeOD): δ = 177.0 ppm, 1C (NH*C*OC(CH_2CH_2)CH_2OH); δ = 98.5 ppm, 1 C (C1); δ = 79.8 ppm, 1 C (OCH_2*C*CH); δ = 76.2 ppm, 1 C (OCH_2C*C*H); δ = 74.7 ppm, 1 C (C5); δ = 70.4, 1 C (C3); δ = 68.9 ppm, 1 C (C4); δ = 66.7 ppm, 1 C (NHCOC(CH_2CH_2)*C*H$_2$OH); δ = 62.6 ppm, 1 C (C6); δ = 55.1 ppm, 1 C (O*C*H$_2$CCH); δ = 54.4 ppm, 1 C (C2); δ = 26.4 ppm, 1 C (NHCO*C*(CH_2CH_2)CH_2OH); δ = 13.4 ppm, 1 C (NHCOC(*C*H$_2$CH$_2$)CH_2OH); δ = 13.2 ppm, 1 C (NHCOC(CH_2*C*H$_2$)CH_2OH).

R_f = 0.33 with 15% MeOH in DCM.

HR ESI-MS for $C_{14}H_{21}NO_7$: m·z^{-1}(M+Na$^+$)$_{calc}$ = 338.122; m·z^{-1}(M+Na$^+$)$_{obs}$ = 338.121.


1.0
3.0
1.0
1.0
5.1
0.8
2.1
2.1
10
9
8
7
6
5
4
3
2
1
0
δ(¹H) [ppm]

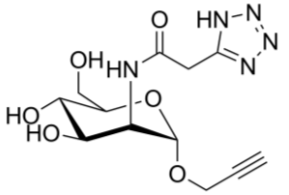
481


176.97
98.47
79.79
76.18
74.73
70.35
68.89
66.71
62.59
55.07
54.41
26.42
13.36
13.19
200
180
160
140
120
100
80
60
40
20
0
δ(¹³C) [ppm]

482

483

Propargyl-2-deoxy-2-2'-(tetrazolidin-5'-yl)acetamido-α-D-mannopyranoside



5.7

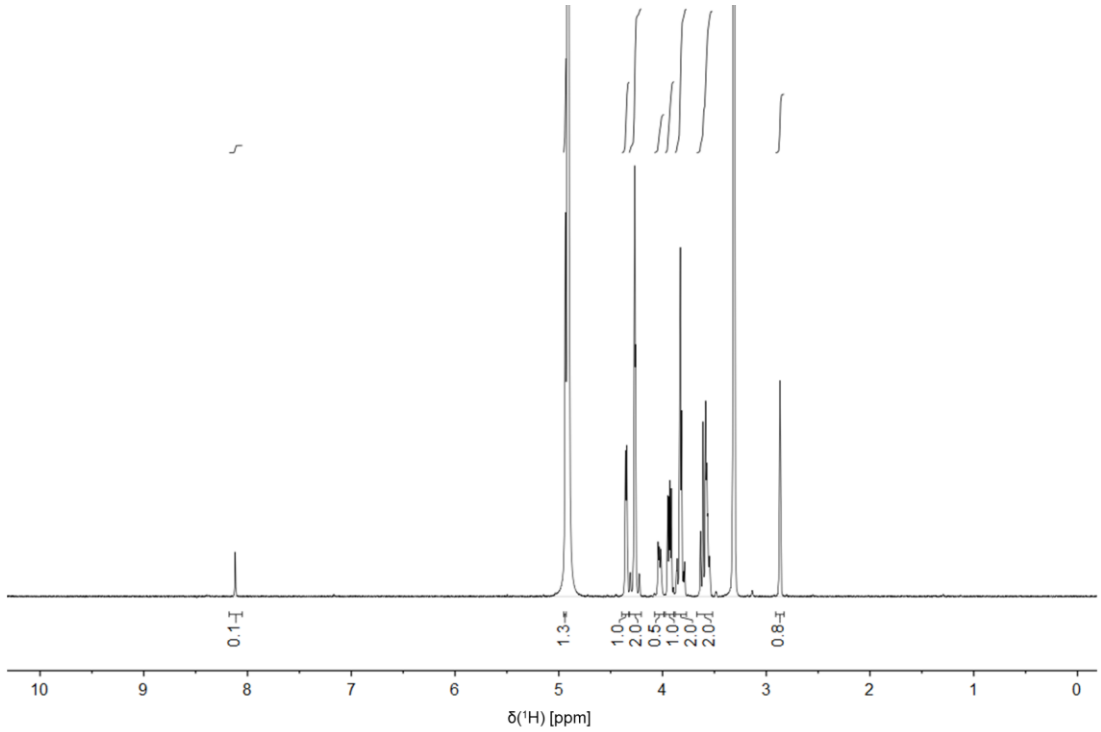
2-(tetrazolidin-5-yl)acetic acid (59 mg, 0.46 mmol, Santa Cruz Biotechnology) was dissolved in DMF (1.2 ml). Subsequently, PyBOP (240 mg, 0.46 mmol) and DIPEA (160 µl, 0.92 mmol) were added and mixture was stirred for 10 min at room temperature. **4** (50 mg, 0.230 mmol) was added and the mixture was stirred overnight at room temperature. Progress of the reaction was monitored by analytical TLC (20% MeOH in DCM). Following quenching with MeOH (1 ml), solvents were removed *in vacuo*. The residue was purified via reversed-phase column chromatography (gradient: elution with H_2O). Residual impurities were removed via preparative HPLC (gradient: H_2O for 10 min, from 0 to 20% acetonitrile in H_2O in 30 min, from 20 to 50% acetonitrile in H_2O in 10 min, from 50 to 100% acetonitrile in H_2O in 5 min and acetonitrile for 5 min at 3.2 $ml \cdot min^{-1}$) to yield **5.7** as a white solid (16 mg, 49 µmol, 21 %).

^{1}H NMR (400.0 MHz, MeOD): δ = 8.11 ppm, s, 1 H (N*H*COCH$_2$N$_4$H); δ = 4.94 ppm, s, 1 H (H1); δ = 4.25 ppm, d, 1 H, J = 4.7 Hz (H2); δ = 4.27 ppm, m, 2 H (OC*H2*CCH); δ = 4.03 ppm, m, 2 H (NHCOC*H*$_2$N$_4$H); δ = 3.93 ppm, dd, 1 H, J = 4.7, 8.9 Hz (H3); δ = 3.83 ppm, m, 2 H (H6a/b); δ = 3.61 ppm, m, 1 H (H4); δ = 3.58 ppm, m, 1 H (H5); δ = 2.87 ppm, m, 1 H (OCH$_2$CC*H*).

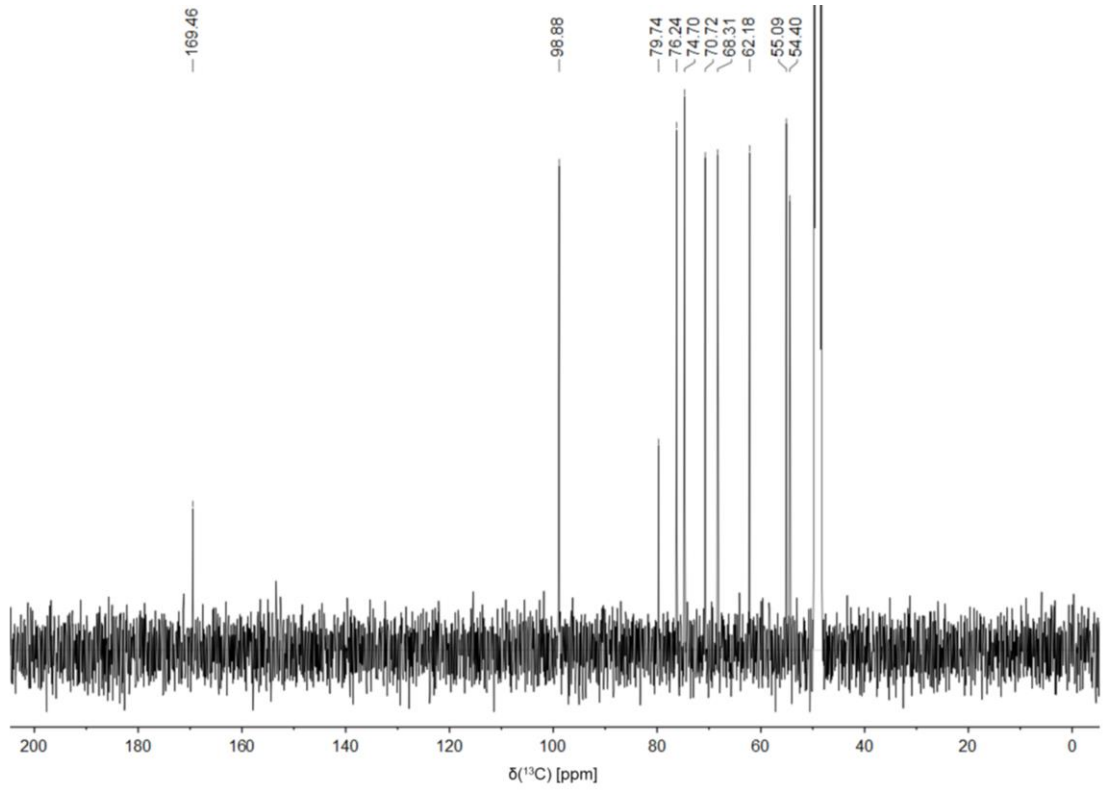
^{13}C NMR (100.6 MHz, MeOD): δ = 169.5 ppm, 1C (NH*C*OCH$_2$N$_4$H); δ = 98.9 ppm, 1 C (C1); δ = 79.7 ppm, 1 C (OCH$_2$*C*CH); δ = 76.2 ppm, 1 C (OCH$_2$C*C*H); δ = 74.7 ppm, 1 C (C5); δ = 70.7 ppm, 1 C (C3); δ = 68.3 ppm, 1 C (C4); δ = 62.2 ppm, 1 C (C6); δ = 55.1, 1 C (O*C*H$_2$CCH); δ = 54.4 ppm, 1 C (C2); δ = 31.0 ppm, 1 C (NHCO*C*H$_2$N$_4$H).

R_f = 0.06 with 20% MeOH in DCM.

HR ESI-MS for $C_{12}H_{17}N_5O_6$: $m \cdot z^{-1}(M+Na^+)_{calc}$ = 350.108; $m \cdot z^{-1}(M+Na^+)_{obs}$ = 350.109.

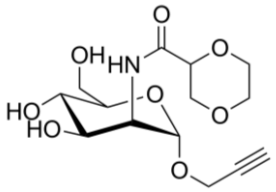

0.1
1.3
1.0
2.0
0.5
1.0
2.0
2.0
0.8
10
9
8
7
6
5
4
3
2
1
0
δ(¹H) [ppm]

507


169.46
98.88
79.74
76.24
74.70
70.72
68.31
62.18
55.09
54.40
200
180
160
140
120
100
80
60
40
20
0
δ(¹³C) [ppm]

508

Propargyl-2-deoxy-2-1',4'-dioxane-2'-carboxamido-α-D-mannopyranoside



5.8

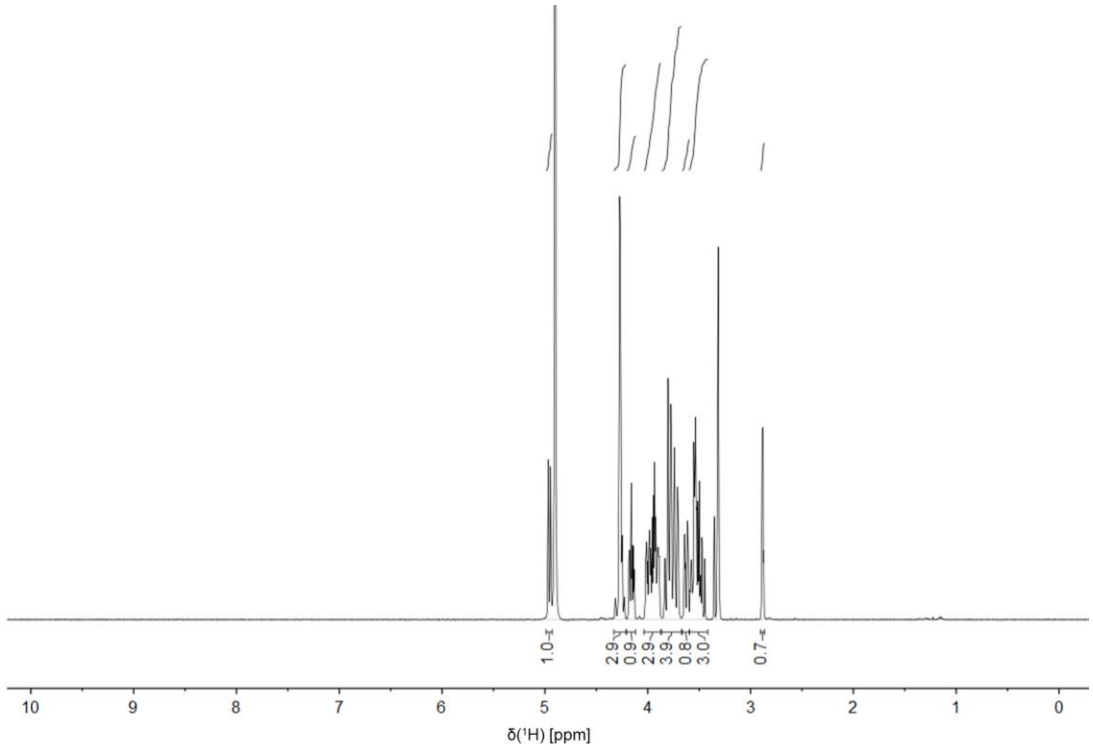
1,4-dioxane-2-carboxylic acid (40 mg, 306 µmol, Santa Cruz Biotechnology) was dissolved in DMF (760 µl). Subsequently PyBOP (158 mg, 306 µmol) and DIPEA (110 µl, 612 µmol) were added and the mixture was stirred for 10 min at room temperature. **4** (33 mg, 152 µmol) was added and the mixture was stirred overnight at room temperature. Progress of the reaction was monitored by analytical TLC (10% MeOH in DCM). Solvents were removed *in vacuo* and the residue was purified via column chromatography (gradient: hexane, DCM, 5 % MeOH in DCM and elution with 10 % MeOH in DCM). Residual impurities were removed via revers-phase column chromatography (gradient: H_2O, 5% MeOH in H_2O and elution at 10% MeOH in H_2O) to yield **5.8** as a white solid (20 mg, 60 µmol, 40%).

^{1}H NMR (400 MHz, MeOD: δ = 4.95 ppm, dd, 1 H, J = 1.0, 8.4 Hz (H1); δ = 4.27 ppm, m, 2H (OC*H*$_2$CCH); δ = 4.25 ppm, m, 1H (H2); δ = 4.15 ppm, m, 1 H (NHCOC*H*CH$_2$OCH$_2$CH$_2$O); δ = 3.99 ppm, m, 1 H (NHCOCHC*H*$_2$OCH$_2$CH$_2$O); δ = 3.94 ppm, m, 1 H (H3); δ = 3.90 ppm, m, 1 H (NHCOCHCH$_2$OC*H*$_2$CH$_2$O); δ = 3.79 ppm, m, 3 H (H6a/b, NHCOCHCH$_2$OC*H*$_2$CH$_2$O); δ = 3.72 ppm, m, 1 H (NHCOCHCH$_2$OCH$_2$C*H*$_2$O); δ = 3.62 ppm, m, 1H (NHCOCHCH$_2$OCH$_2$C*H*$_2$O); δ = 3.54 ppm, m, 1H (H5); δ = 3.52 ppm, m, 1 H (H4); δ = 3.47 ppm, m, 1 H (NHCOCHC*H*$_2$OCH$_2$CH$_2$O); δ = 2.87 ppm, m, 1 H (OCH$_2$CC*H*)

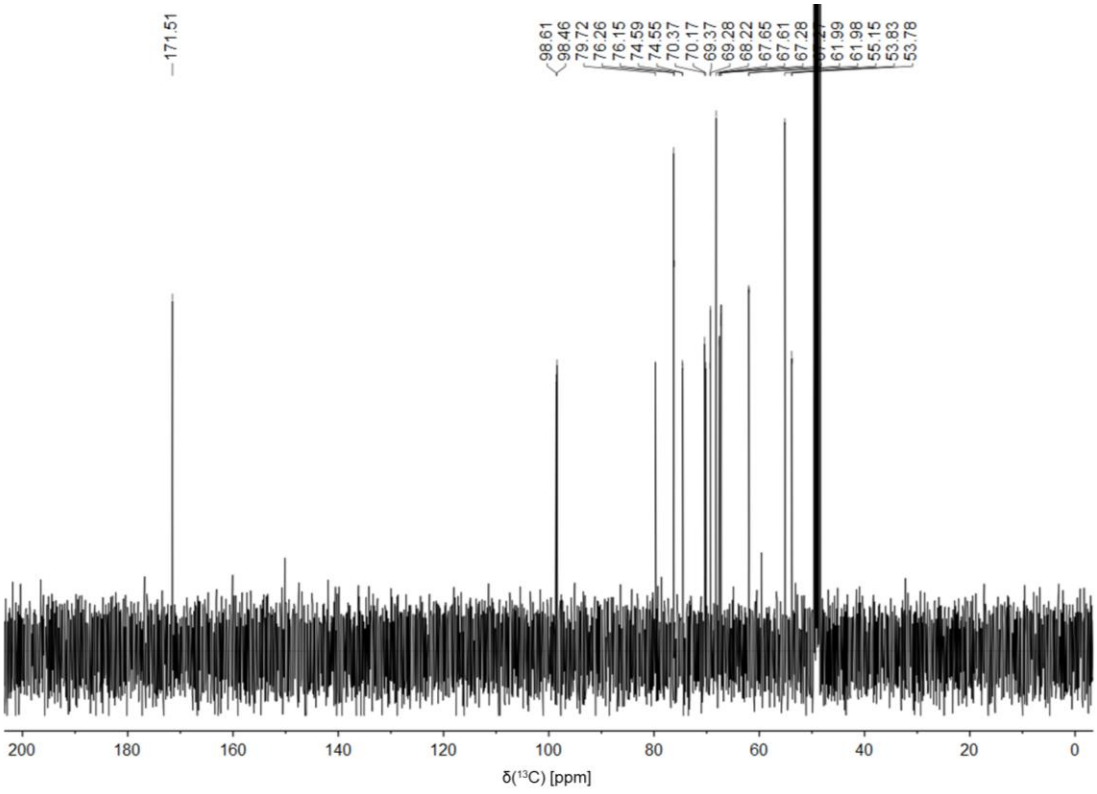
^{13}C NMR (100 MHz, MeOD): δ = 171.5 ppm, 1 C (NH*C*OCHCH$_2$OCH$_2$CH$_2$O) ; δ = 98.8 ppm, d, 1 C, J = 16.5 Hz (C1); δ = 79.7 ppm, 1 C (OCH$_2$*C*CH); δ = 76.3 ppm, m, 1 C (NHCO*C*HCH$_2$OCH$_2$CH$_2$O); δ = 76.2 ppm, 1 C (OCH$_2$C*C*H); δ = 74.6 ppm, d, 1C, J = 4.2 Hz (C5); δ = 70.3 ppm, d, J = 20.0 Hz (C3); δ = 69.4 ppm, d, 1C, J = 8.9 Hz (NHCOCH*C*H$_2$OCH$_2$CH$_2$O); δ = 68.2 ppm, 1C (C4); δ = 67.6 ppm, d, 1C, J = 3.9 Hz (NHCOCHCH$_2$OC*H*$_2$CH$_2$O); δ = 67.3 ppm, d, 1C, J = 1.6 Hz (NHCOCHCH$_2$OCH$_2$C*H*$_2$O); δ = 62.0 ppm, d, 1C, J = 1.3 Hz (C6); δ = 55.2 ppm, 1C (OCH$_2$CCH); δ = 53.81 ppm, d, 1C, J = 4.9 Hz (C2)

R_f = 0.19 with 10% in MeOH in DCM.

HR ESI-MS for $C_{14}H_{21}NO_8$: $m{\cdot}z^{-1}(M+Na^+)_{calc}$ = 354.117; $m{\cdot}z^{-1}(M+Na^+)_{obs}$ = 354.114.


1.0
2.9
0.9
2.9
3.9
0.8
3.0
0.7
10
9
8
7
6
5
4
3
2
1
0
δ(¹H) [ppm]

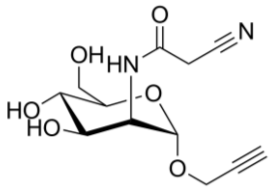
536


171.51
98.61
98.46
79.72
76.26
76.15
74.59
74.55
70.37
70.17
69.37
69.28
68.22
67.65
67.61
67.28
61.99
61.98
55.15
53.83
53.78
200
180
160
140
120
100
80
60
40
20
0
δ(¹³C) [ppm]

537

538

Propargyl-2-deoxy-2-2'-cyanoacetamido-α-D-mannopyranoside



5.9

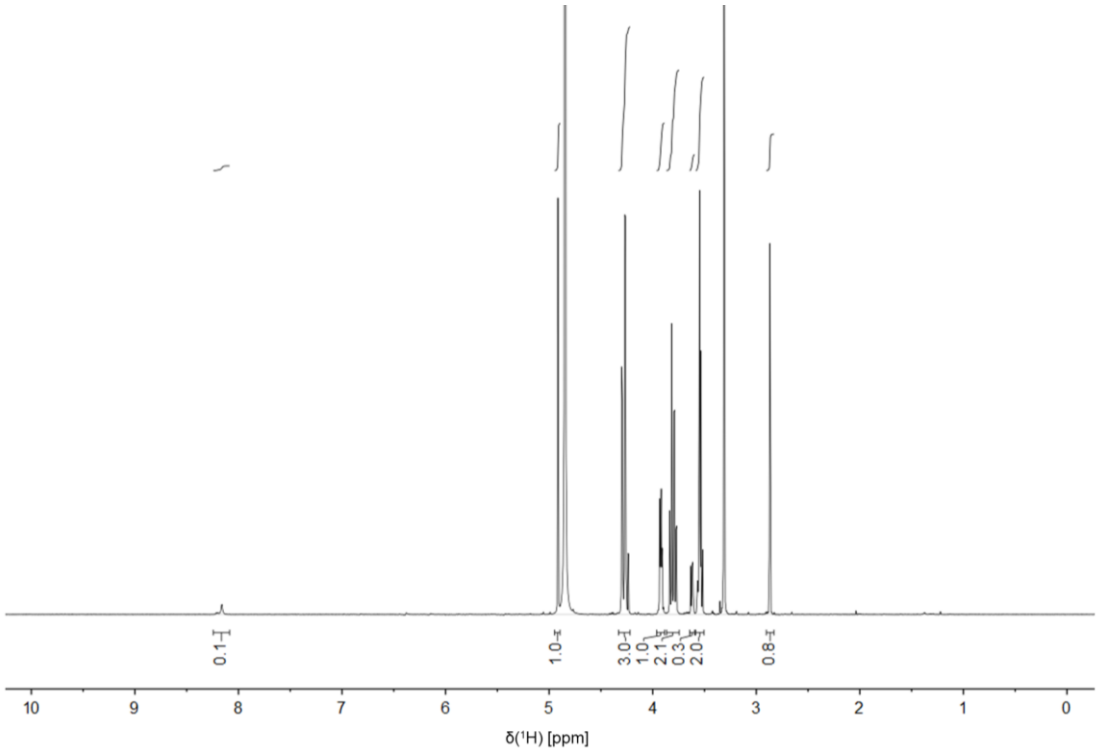
2-cyanoacetic acid (39.0 mg, 0.46 mmol) was dissolved in DMF (1.2 ml). Subsequently PyBOP (240 mg, 0.46 mmol) and DIPEA (160 µl, 0.92 mmol) were added and the mixture was stirred for 10 min at room temperature. **4** (50 mg, 0.230 mmol) was added and the mixture was stirred overnight at room temperature. Progress of the reaction was monitored by analytical TLC (10% MeOH in DCM). Solvents were removed *in vacuo* and the residue was purified via column chromatography (gradient: hexane, DCM, 5 % MeOH in DCM and elution with 20 % MeOH in DCM). Residual impurities were removed via preparative HPLC (gradient: H_2O for 10 min, from 0 to 20% acetonitrile in H_2O in 30 min, from 20 to 50% acetonitrile in H_2O in 10 min, from 50 to 100% acetonitrile in H_2O in 5 min and acetonitrile for 5 min at 3.2 ml·min^{-1}) to yield **5.9** as a white solid (10.2 mg, 36 µmol, 16%).

^{1}H NMR (600.0 MHz, MeOD): δ = 8.16 ppm, s, 1 H (N*H*COCH$_2$CN); δ = 4.91 ppm, d, 1 H, J = 1.1 Hz (H1); δ = 4.29 ppm, m, 1 H (H2); δ = 4.27 ppm, m, 2 H (OC*H2*CCH); δ = 3.92 ppm, dd, 1 H, J = 4.8, 9.1 Hz (H3); δ = 3.81 ppm, m, 2 H (H6a/b); δ = 3.62 ppm, m, 2 H (NHCOC*H*$_2$CN); δ = 3.54 ppm, m, 2 H (H4, H5); δ = 2.87 ppm, t, 1 H, J = 2.3 Hz (OCH$_2$CC*H*).

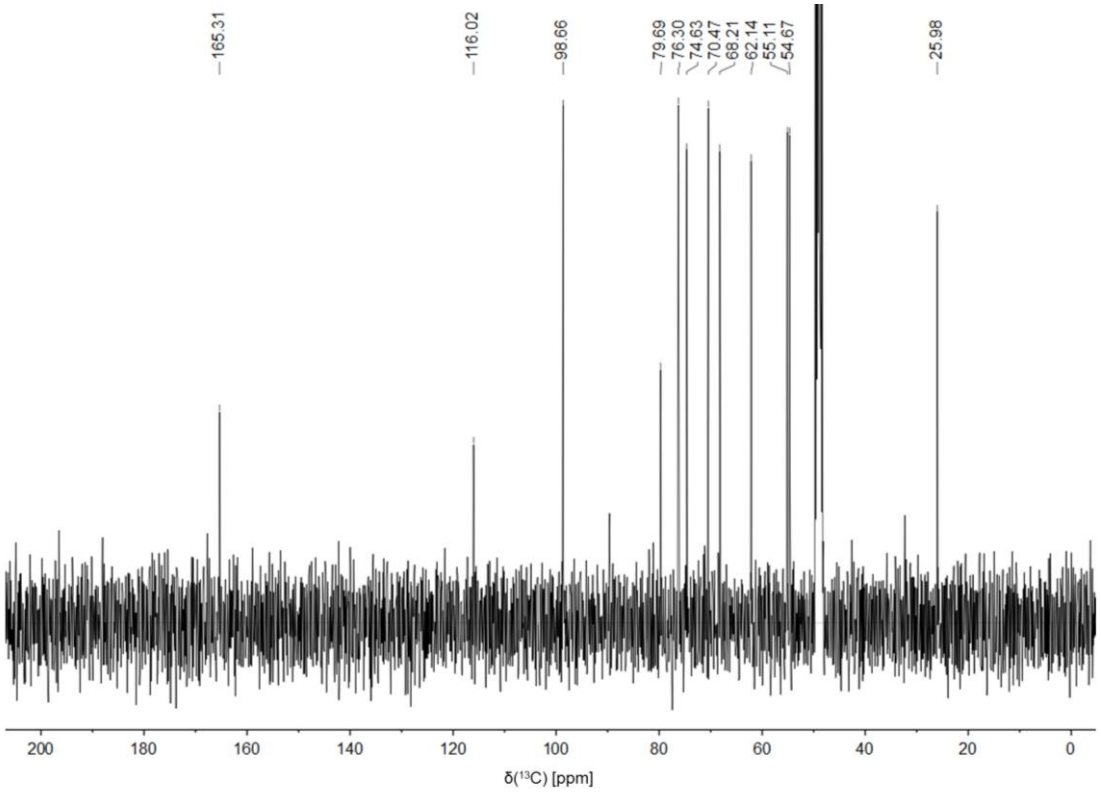
^{13}C NMR (100.6 MHz, MeOD): δ = 165.3 ppm, 1 C (NH*C*OCH$_2$CN); δ = 116.0 ppm, 1 C (NHCOCH$_2$*C*N); δ = 98.7 ppm, 1 C (C1); δ = 79.7 ppm, 1 C (OCH$_2$*C*CH); δ = 76.3 ppm, 1 C (OCH$_2$C*C*H); δ = 74.6 ppm, 1 C (C5); δ = 70.47, 1 C (C3); δ = 68.2 ppm, 1 C (C4); δ = 62.1 ppm, 1 C (C6); δ = 55.1, 1 C (O*C*H$_2$CCH); δ = 54.7 ppm, 1 C (C2); δ = 26.0 ppm, 1 C (NHCO*C*H$_2$CN).

R_f = 0.25 with 10% in MeOH in DCM.

HR ESI-MS for $C_{12}H_{16}N_2O_6$: m·z^{-1}(M+Na$^+$)$_{calc}$ = 307.091; m·z^{-1}(M+Na$^+$)$_{obs}$ = 307.096.


0.1
1.0
3.0
1.0
2.1
0.3
2.0
0.8
10
9
8
7
6
5
4
3
2
1
0
δ(¹H) [ppm]

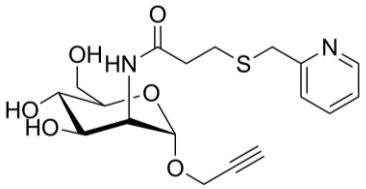
560


165.31
116.02
98.66
79.69
76.30
74.63
70.47
68.21
62.14
55.11
54.67
25.98
200
180
160
140
120
100
80
60
40
20
0
δ(¹³C) [ppm]

561

562

Propargyl-2-deoxy-2-3'-((pyridin-2'-ylmethyl)thio)propanamido-α-D-mannopyranoside



5.10

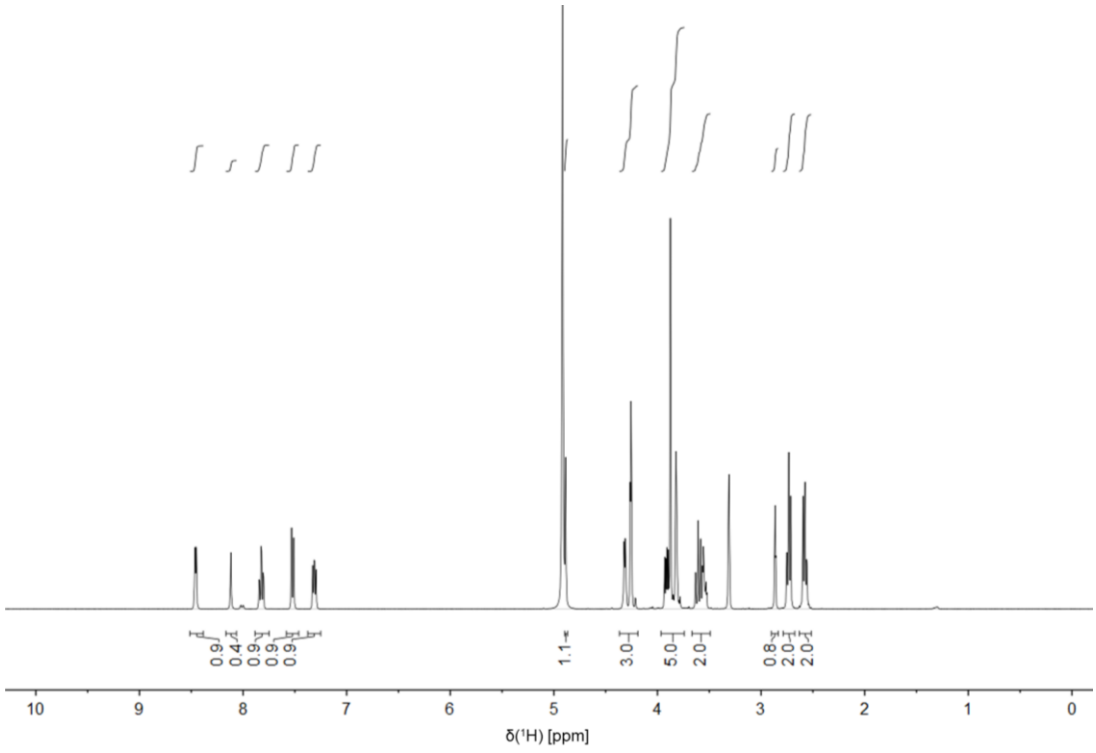
3-((Pyridin-2-ylmethyl)thio)propanoic acid (42.0 mg, 210 µmol, Enamine) was dissolved in DMF (600 µl). Subsequently PyBOP (110 mg, 210 µmol) and DIPEA (80 µl, 430 µmol) were added and the mixture was stirred for 10 min at room temperature. **4** (23 mg, 110 µmol) was added and the mixture was stirred overnight at room temperature. Progress of the reaction was monitored by analytical TLC (15% MeOH in DCM). The reaction was quenched with MeOH (1 ml) and after addition of 1 M NaOH (600 µl, 600 µmol) solvents were removed *in vacuo*. The residue was purified via preparative HPLC (gradient: H_2O for 10 min, from 0 to 20% acetonitrile in H_2O in 30 min, from 20 to 50% acetonitrile in H_2O in 10 min, from 50 to 100% acetonitrile in H_2O in 5 min and acetonitrile for 5 min at 3.2 ml·min^{-1}) to yield **5.10** as a white solid (26.1 mg, 66 µmol, 62%).

^{1}H NMR (400.0 MHz, MeOD): δ = 8.46 ppm, d, 1 H, J = 4.8 Hz ($SCH_2CCHCHCHC\mathit{H}N$); δ = 8.12 ppm, s, 1 H ($N\mathit{H}COCH_2CH_2S$); δ = 7.82 ppm, td, 1 H, J = 1.5, 7.7 Hz ($SCH_2CCHC\mathit{H}CHCHN$); δ = 7.52 ppm, d, 1 H, J = 7.9 Hz ($SCH_2CC\mathit{H}CHCHCHN$); δ = 7.31 ppm, dd, 1 H, J = 5.2, 7.4 Hz ($SCH_2CCHCHC\mathit{H}CHN$); δ = 4.89 ppm, s, 1 H (H1); δ = 4.32 ppm, d, 1 H, J = 5.0 Hz (H2); δ = 4.26 ppm, m, 2 H ($OC\mathit{H2}CCH$); δ = 3.91 ppm, dd, 1 H, J = 4.8, 9.3 Hz (H3); δ = 3.87 ppm, s, 2 H ($SC\mathit{H}_2CCHCHCHCHN$); δ = 3.81 ppm, m, 2 H (H6a/b); δ = 3.61 ppm, m, 1 H (H4); δ = 3.54 ppm, m, 1 H (H5); δ = 2.86 ppm, t, 1 H, J = 2.3 Hz ($OCH_2CC\mathit{H}$); δ = 2.74 ppm, m, 2 H ($NHCOC\mathit{H}_2CH_2S$); δ = 2.58 ppm, m, 2 H ($NHCOCH_2C\mathit{H}_2S$).

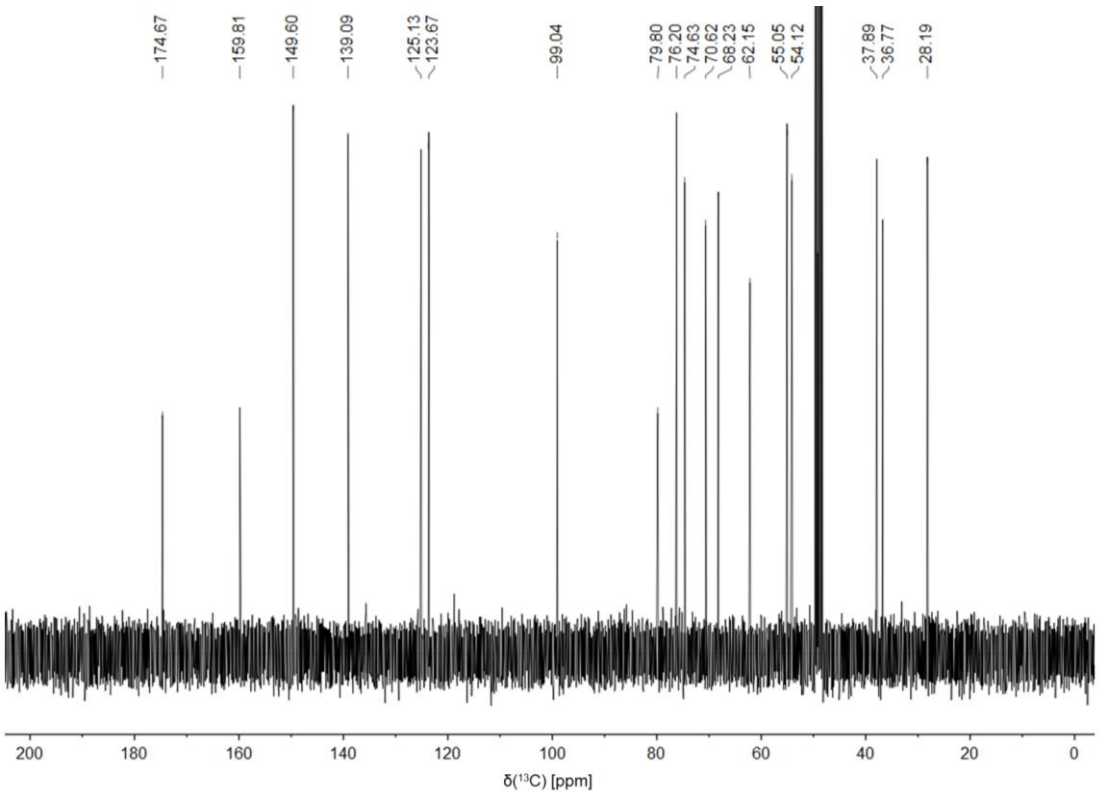
^{13}C NMR (100.6 MHz, MeOD): δ = 174.7 ppm, 1 C ($NHC\mathit{O}CH_2CH_2S$); δ = 159.8 ppm, 1 C ($SCH_2\mathit{C}CHCHCHCHN$); δ = 149.6 ppm, 1 C ($SCH_2CCHCHCH\mathit{C}HN$); δ = 139.1 ppm, 1 C ($SCH_2CCH\mathit{C}HCHCHN$); δ = 125.1 ppm, 1 C ($SCH_2C\mathit{C}HCHCHCHN$); δ = 123.7 ppm, 1 C ($SCH_2CCHCH\mathit{C}HCHN$); δ = 99.0 ppm, 1 C (C1); δ = 79.8 ppm, 1 C ($OCH_2\mathit{C}CH$); δ = 76.2 ppm, 1 C ($OCH_2C\mathit{C}H$); δ = 74.6 ppm, 1 C (C5); δ = 70.62, 1 C (C3); δ = 68.2 ppm, 1 C (C4); δ = 62.2 ppm, 1 C (C6); δ = 55.1 ppm, 1 C ($O\mathit{C}H_2CCH$); δ = 54.1 ppm, 1 C (C2); δ = 37.9 ppm, 1 C ($S\mathit{C}H_2CCHCHCHCHN$); δ = 36.8 ppm, 1 C ($NHCO\mathit{C}H_2CH_2S$); δ = 28.2 ppm, 1 C ($NHCO\mathit{C}H_2CH_2S$).

R_f = 0.67 with 15% in MeOH in DCM.

HR ESI-MS for $C_{18}H_{24}N_2O_6S$: $m{\cdot}z^{-1}(M+Na^+)_{calc}$ = 419.125; $m{\cdot}z^{-1}(M+Na^+)_{obs}$ = 419.124.


0.9
0.4
0.9
0.9
0.9
1.1
3.0
5.0
2.0
0.8
2.0
2.0
δ(¹H) [ppm]

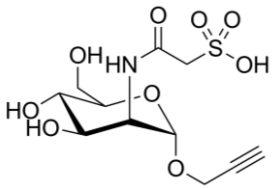
591


174.67
159.81
149.60
139.09
125.13
123.67
99.04
79.80
76.20
74.63
70.62
68.23
62.15
55.05
54.12
37.89
36.77
28.19
δ(¹³C) [ppm]

592

593

Propargyl-2-deoxy-2-*N* -2'-oxoethanesulfonic acid -α-D-mannopyranoside



5.11

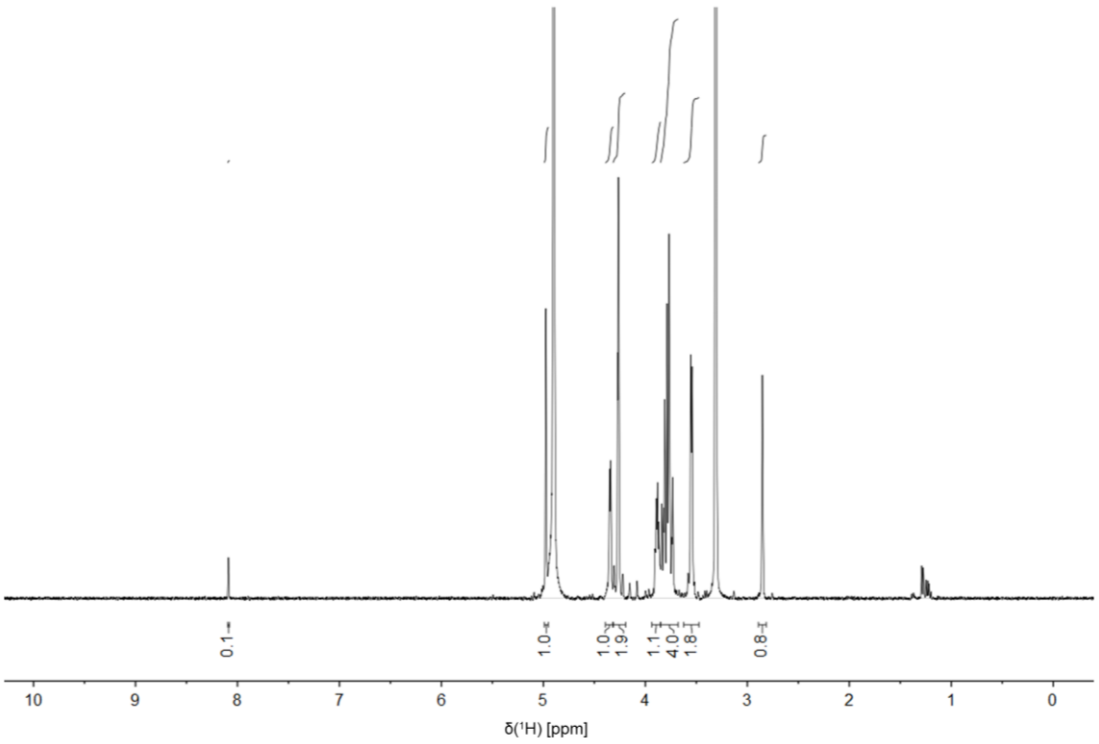
2-Sulfoacetic acid (65 mg, 460 µmol) was dissolved in DMF (1.2 ml). Subsequently, PyBOP (240 mg, 460 µmol) and DIPEA (160 µl, 920 µmol) were added and the mixture was stirred for 10 min at room temperature. **4** (45 mg, 230 µmol) was added and the mixture was stirred overnight at room temperature. Progress of the reaction was monitored by analytical TLC (20% MeOH in DCM). After the addition of 1 M NaOH (1.4 ml, 1.4 mmol) solvents were removed *in vacuo.* The residue was purified via reversed-phase column chromatography (elution with H_2O). Residual impurities were removed via preparative HPLC (gradient: H_2O for 10 min, from 0 to 100% acetonitrile in H_2O in 40 min, and acetonitrile for 10 min at 1.0 ml·min^{-1}) to yield **5.11** as a white solid (18 mg, 53 µmol, 23%).

^{1}H NMR (600.0 MHz, MeOD): δ = 8.09 ppm, s, 1 H (N*H*COCH$_2$SO$_3$H); δ = 4.98 ppm, s, 1 H (H1); δ = 4.35 ppm, d, 1 H, J = 4.2 Hz (H2); δ = 4.27 ppm, m, 2 H (OC*H2*CCH); δ = 3.89 ppm, dd, 1 H, J = 4.5, 9.2 Hz (H3); δ = 3.78 ppm, m, 3 H (NHCOC*H$_2$*SO$_3$H, H6a/b); δ = 3.55 ppm, m, 2 H (H4, H5); δ = 2.85 ppm, t, 1 H, J = 2.4 Hz (OCH$_2$CC*H*).

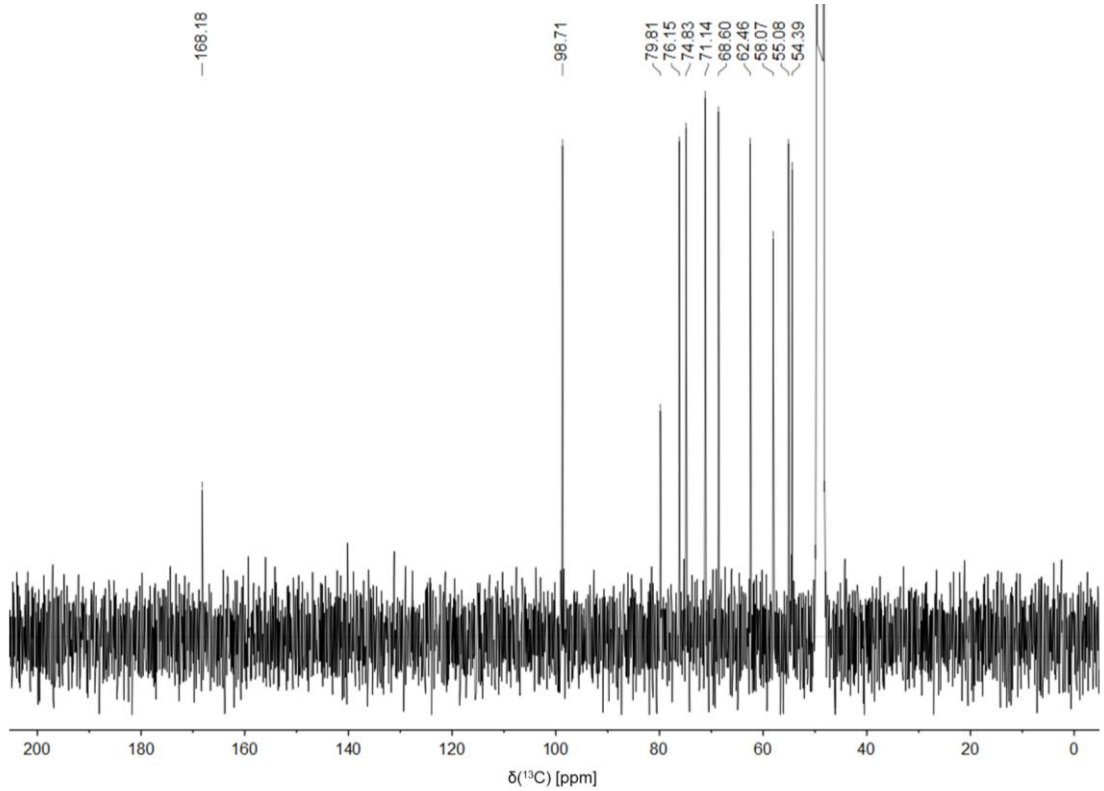
^{13}C NMR (100.6 MHz, MeOD): δ = 168.2 ppm, 1 C (NH*C*OCH$_2$SO$_3$H); δ = 98.7 ppm, 1 C (C1); δ = 79.8 ppm, 1 C (OCH$_2$*C*CH); δ = 76.2 ppm, 1 C (OCH$_2$C*C*H); δ = 74.8 ppm, 1 C (C5); δ = 71.1 ppm, 1 C (C3); δ = 68.6 ppm, 1 C (C4); δ = 62.5 ppm, 1 C (C6); δ = 58.1 ppm, 1 C (NHCO*C*H$_2$SO$_3$H); δ = 55.1 ppm, 1 C (O*C*H$_2$CCH); δ = 54.4 ppm, 1 C (C2).

R_f = 0.04 with 20% in MeOH in DCM.

HR ESI-MS for $C_{11}H_{17}NO_9S$: m·z^{-1}(M-H$^+$)$_{calc}$ = 338.054; m·z^{-1}(M-H$^+$)$_{obs}$ = 338.058.


0.1
1.0
1.0
1.9
1.1
4.0
1.8
0.8
10
9
8
7
6
5
4
3
2
1
0
δ(¹H) [ppm]

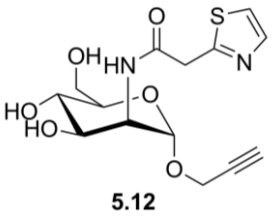
614


168.18
98.71
79.81
76.15
74.83
71.14
68.60
62.46
58.07
55.08
54.39
200
180
160
140
120
100
80
60
40
20
0
δ(¹³C) [ppm]

615

616

Propargyl-2-deoxy-2- 2'-(thiazol-2'-yl)acetamido-α-D-mannopyranoside



5.12

2-(Thiazol-2-yl)acetic acid (50.0 mg, 350 µmol) was dissolved in DMF (0.9 ml). Subsequently, PyBOP (180 mg, 350 µmol) and DIPEA (120 µl, 700 µmol) were added and the mixture was stirred for 10 min at room temperature. 16 h after the addition of **4** (38 mg, 180 µmol), more PyBOP (180 mg, 350 µmol), DIPEA (120 µl, 700 µmol) and 2-(thiazol-2-yl)acetic acid (50 mg, 350 µmol) were added. The mixture was stirred overnight at room temperature and progress of the reaction was monitored by analytical TLC (10% MeOH in DCM). After the addition of 1 M NaOH (1.4 ml, 1.4 mmol) solvents were removed *in vacuo.* The residue was purified via reversed-phase column chromatography (elution with H_2O). Residual impurities were removed via preparative HPLC (gradient: H_2O for 10 min, from 0 to 20% acetonitrile in H_2O in 30 min, from 20 to 50% acetonitrile in H_2O in 10 min, from 50 to 100% acetonitrile in H_2O in 5 min and acetonitrile for 5 min at 3.2 ml·min^{-1}) to yield **5.12** as a white solid (5.2 mg, 15 µmol, 9%).

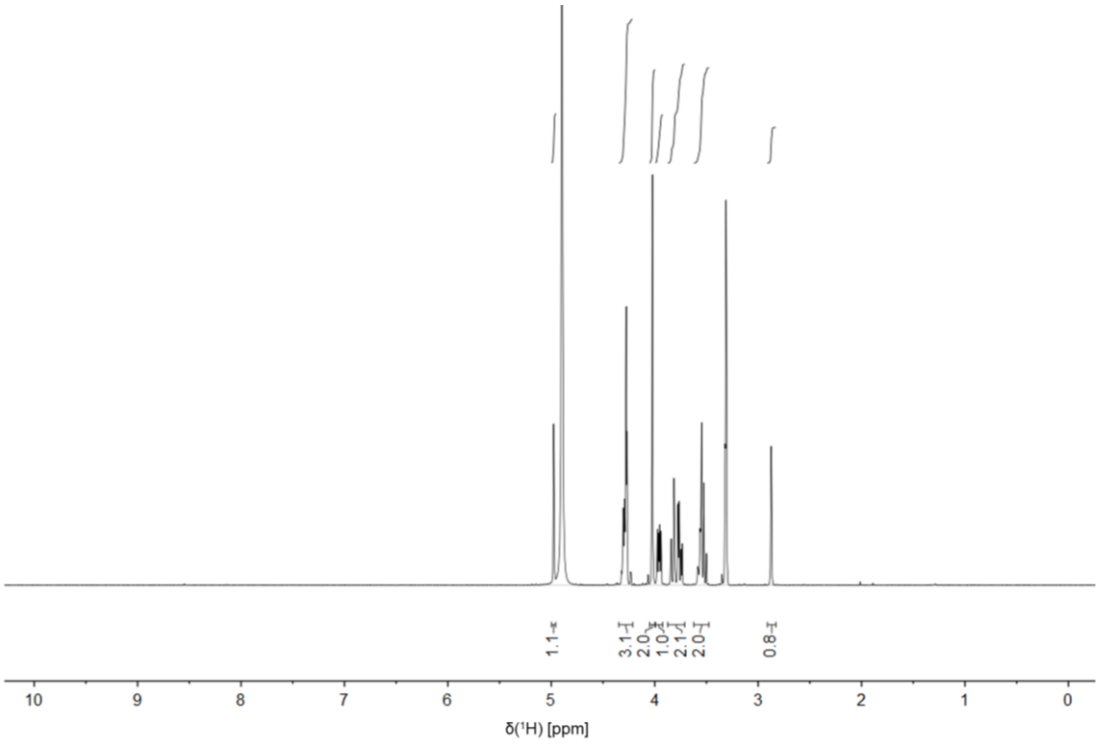
^{1}H NMR (600.0 MHz, MeOD): δ = 4.97 ppm, d, 1 H, J = 1.2 Hz (H1); δ = 4.30 ppm, dd, 1 H, J = 1.3, 4.8 Hz (H2); δ = 4.28 ppm, m, 2 H (OC*H2*CCH); δ = 4.02 ppm, s, 2 H (NHCOC*H*$_2$CNCCS); δ = 3.96 ppm, dd, 1 H, J = 4.8, 8.8 Hz (H3); δ = 3.83 ppm, m, 1 H (H6a); δ = 3.75 ppm, m, 1 H (H6b); δ = 3.57 ppm, m, 1 H (H5); δ = 3.53 ppm, m, 1 H (H4); δ = 2.87 ppm, t, 1 H, J = 2.4 Hz (OCH$_2$CC*H*).

^{13}C NMR (100.6 MHz, MeOD): δ = 175.5 ppm, 1 C (NH*C*OCH$_2$CNCCS); δ = 98.6 ppm, 1 C (C1); δ = 79.7 ppm, 1 C (OCH$_2$*C*CH); δ = 76.2 ppm, 1 C (OCH$_2$C*C*H); δ = 74.6 ppm, 1 C (C5); δ = 70.4 ppm, 1 C (C3); δ = 68.4 ppm, 1 C (C4); δ = 62.6 ppm, 1 C (NHCO*C*H$_2$CNCCS); δ = 62.1 ppm, 1 C (C6); δ = 55.1 ppm, 1 C (O*C*H$_2$CCH); δ = 53.8 ppm, 1 C (C2).

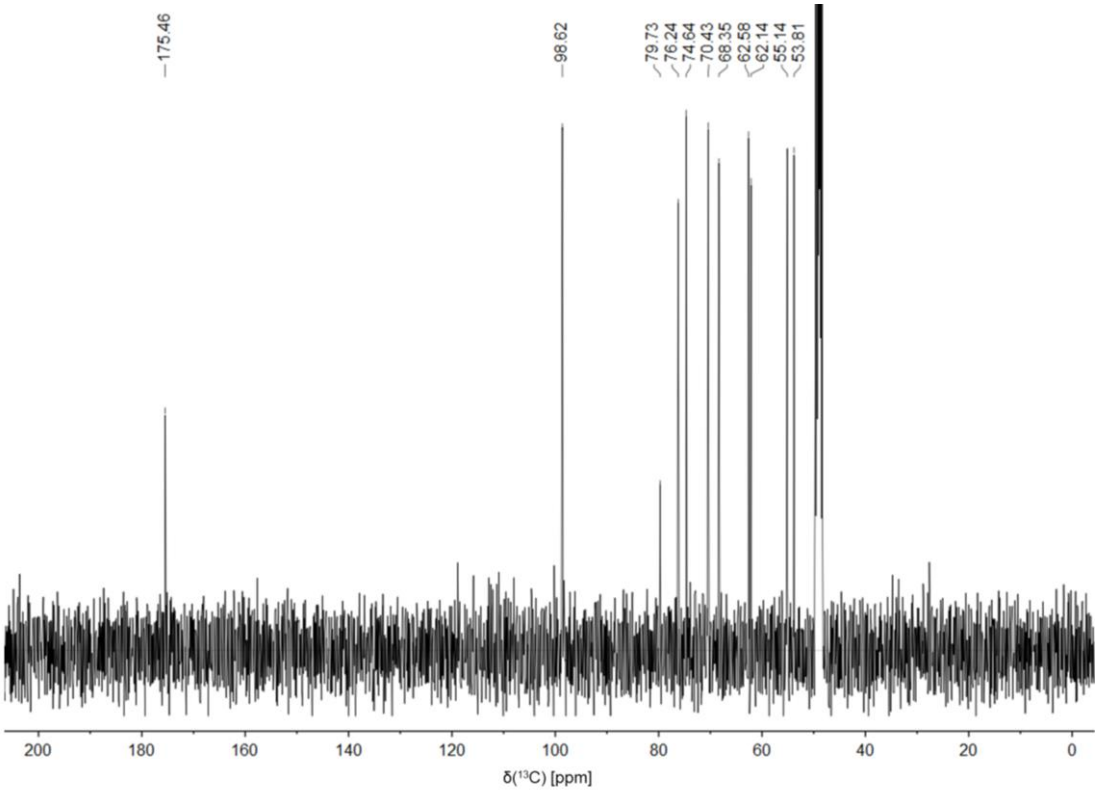
The aromatic carbon atoms of the thiazolyl were not detected in the conducted ^{13}C NMR experiments.

R_f = 0.24 with 10% in MeOH in DCM.

HR ESI-MS for $C_{14}H_{18}N_2O_6S$: m·z^{-1}(M+Na$^+$)$_{calc}$ = 365.078; m·z^{-1}(M+Na$^+$)$_{obs}$ = 365.076.


1.1
3.1
2.0
1.0
2.1
2.0
0.8
10
9
8
7
6
5
4
3
2
1
0
δ(¹H) [ppm]

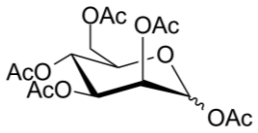
641


175.46
98.62
79.73
76.24
74.64
70.43
68.35
62.58
62.14
55.14
53.81
200
180
160
140
120
100
80
60
40
20
0
δ(¹³C) [ppm]

642

643

1,2,3,4,6-penta-O-acetyl-D-mannopyranose



6

6 was prepared as previously published (*21*). Mannose (5.0 g, 28 mmol) and acetic anhydride (42 ml, 440 mmol) were dissolved in pyridine (100 ml). The reaction mixture was stirred over night at 50°C. Progress of the reaction was monitored by TLC in toluene:ethyl acetate (2:1). Solvents were evaporated *in vacuo* and the residue was taken up in chloroform (250 ml). The organic phase was extracted with 1 M HCl, saturated $NaHCO_3$ and H_2O. Subsequently, the organic phase was dried with $MgSO_4$. Solvents were evaporated in *in vacuo* and the residue was purified via column chromatography (toluene:ethyl acetate (6:1)) to afford an α/β-anomer mixture of **6** (7.20 g, 18.45 mmol, 80 %) as a white solid.

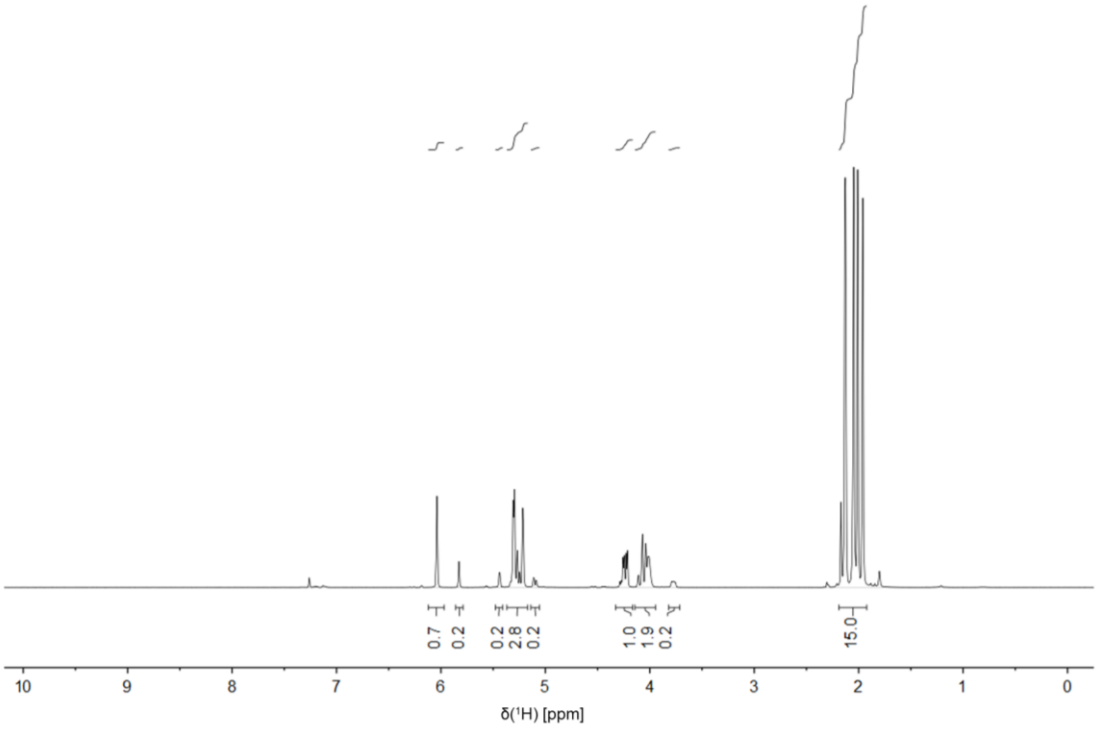
^{1}H NMR (400.0 MHz, $CDCl_3$, α-anomer): δ = 6.05 ppm, m, 1 H (H1); δ = 5.30 ppm, m, 2 H (H3, H4); δ = 5.22, m, 1H (H2); δ = 4.23 ppm, dd, 1 H, J = 4.7, 12.1 Hz (H6a); δ = 4.05, m, 1 H (H6b); δ = 4.01 ppm, m, 1 H (H5); δ = 2.17 - 1.96 ppm, m, 15 H (5 times $OCOCH_3$).

^{13}C NMR (100.6 MHz, $CDCl_3$, α-anomer): δ = 170.6 ppm, 1C ($OCOCH_3$); δ = 170.0, 1C ($OCOCH_3$); δ = 169.7 ppm ($OCOCH_3$); δ = 169.5 ppm, 1C ($OCOCH_3$); δ = 168.1 ppm, 1C ($OCOCH_3$); δ = 90.6 ppm, 1 C (C1); δ = 70.6 ppm, 1 C (C5); δ = 68.8 ppm, 1 C (C3); δ = 68.4 ppm, 1 C (C2); δ = 65.6 ppm, 1 C (C4); δ = 62.1, 1 C (C6); δ = 20.9 ppm, 1 C ($OCOCH_3$); δ = 20.8 ppm, 1 C ($OCOCH_3$); δ = 20.7 ppm, 3 C (three times $OCOCH_3$).

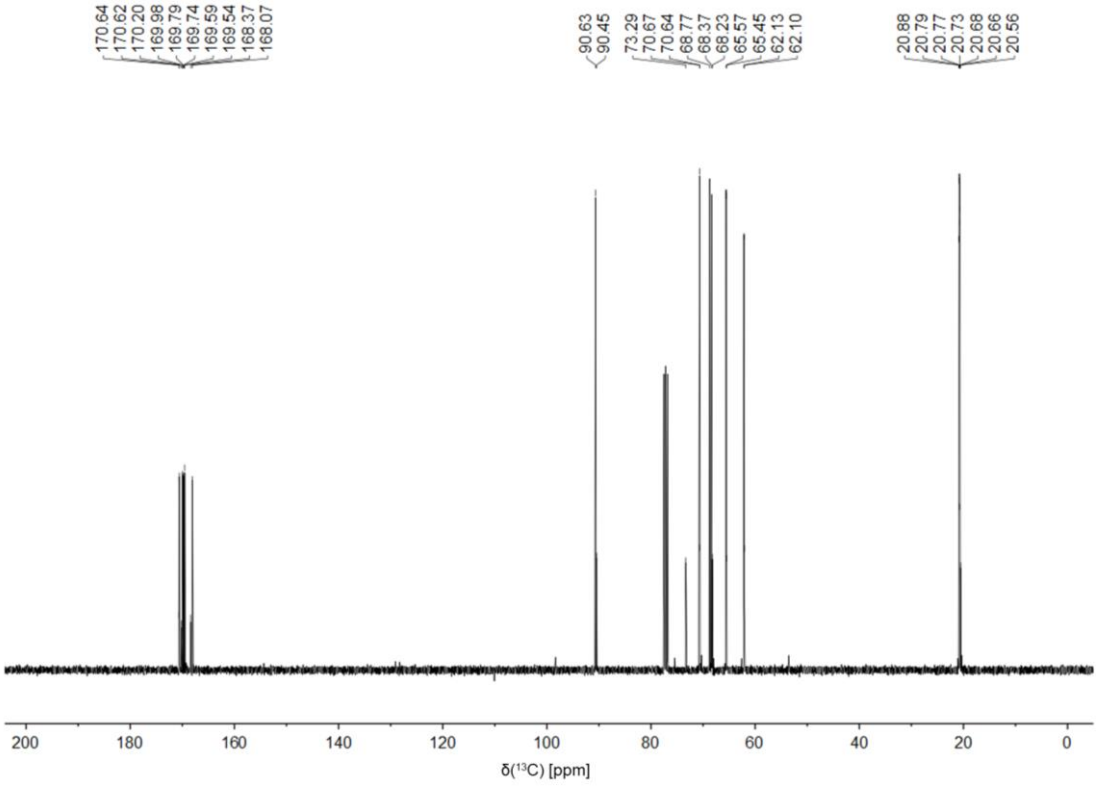
^{1}H NMR (400.0 MHz, $CDCl_3$, β-anomer): δ = 5.83 ppm, m, 1 H (H1); δ = 5.44 ppm, m, 1 H (H2); δ = 5.25 ppm, m, 1 H (H4); δ = 5.10, dd, 1H, J = 2.5, 10.2 Hz (H3); δ = 4.27 ppm, m, 1 H (H6a); δ = 4.05, m, 1 H (H6b); δ = 3.77, m, 1 H (H5); δ = 2.17 - 1.96 ppm, m, 15 H (five times $OCOCH_3$).

^{13}C NMR (100.6 MHz, $CDCl_3$, β-anomer): δ = 170.6 ppm, 1C ($OCOCH_3$); δ = 170.2, 1C ($OCOCH_3$); δ = 169.8 ppm ($OCOCH_3$); δ = 169.6 ppm, 1C ($OCOCH_3$); δ = 168.4 ppm, 1C ($OCOCH_3$); δ = 90.5 ppm, 1 C (C1); δ = 73.3 ppm, 1 C (C5); δ = 70.7 ppm, 1 C (C3); δ = 68.2 ppm, 1 C (C2); δ = 65.5 ppm, 1 C (C4); δ = 62.1, 1 C (C6); δ = 20.8 – 20.6 ppm, 5 C (five times $OCOCH_3$).

R_f = 0.43 with toluene:ethyl acetate (3:1).


0.7
0.2
0.2
2.8
0.2
1.0
1.9
0.2
15.0
10
9
8
7
6
5
4
3
2
1
0
δ(¹H) [ppm]

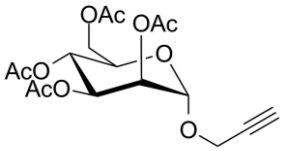
670


170.64
170.62
170.20
169.98
169.79
169.74
169.59
169.54
168.37
168.07
90.63
90.45
73.29
70.67
70.64
68.77
68.37
68.23
65.57
65.45
62.13
62.10
20.88
20.79
20.77
20.73
20.68
20.66
20.56
200
180
160
140
120
100
80
60
40
20
0
δ(¹³C) [ppm]

671

672

Propargyl-2,3,4,6-tetra-O-acetyl-D-mannopyranoside



7

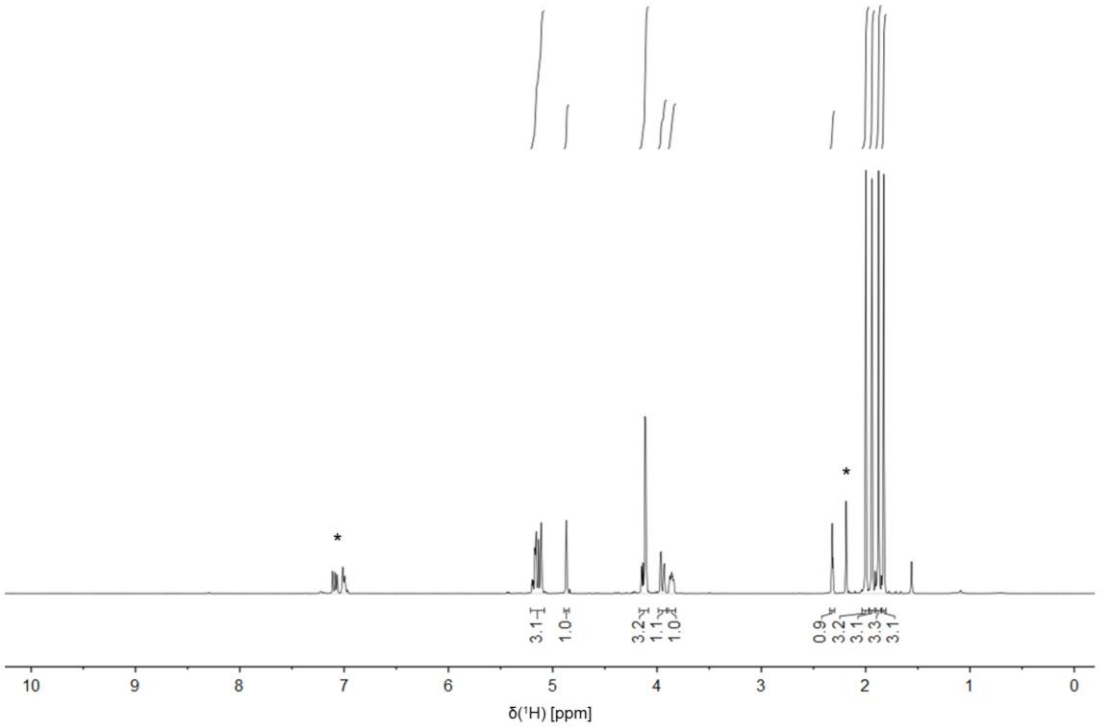
6 (7.20 g, 19 mmol) was dissolved in anhydrous DCM:ether (189 ml, 2:1). The mixture was stirred at 0°C and kept under argon. Propargyl alcohol (4.4 ml, 73.8 mmol) and $BF_3{\cdot}OEt_2$ (4.7 ml, 36.9 mmol) were added and the reaction was allowed to heat up to room temperature and stirred. After 24 h additional propargyl alcohol (4.4 ml, 73.8 mmol) and $BF_3{\cdot}OEt_2$ (4.7 ml, 36.9 mmol) were added at 0°C and the reaction was stirred for another 40 h. Progress of the reaction was monitored by analytical TLC (toluene:ethyl acetate (3:1)). The mixture was diluted in DCM (500 ml) and the organic phase was extracted with saturated $NaHCO_3$ and H_2O. Subsequently, the organic phase was dried with $MgSO_4$. Solvents were evaporated *in vacuo* and the residue was purified via column chromatography (toluene:ethyl acetate (8:1)) to yield 7 (188 mg, 487 µmol, 3%) as a light yellow resin. Starting material **2** that was not converted was recovered.

^{1}H NMR (400.0 MHz, $CDCl_3$): δ = 5.17 ppm, d, 1 H, J = 1.1 Hz (H3); δ = 5.15 ppm, m (H4); δ = 5.11 ppm, m, 1 H (H2); δ = 4.87 ppm, d, 1H, J = 1.3 Hz (H1); δ = 4.16 ppm, m, 1 H (H6a); δ = 4.11 ppm, d, 2 H, J = 2.4 Hz (OC*H*$_2$CCH); δ = 4.27 ppm, dd, 1 H, J = 2.3, 12.2 Hz (H6b); δ = 3.86 ppm, ddd, 1 H, J = 2.3, 5.3, 9.3 Hz (H5); δ = 2.32, t, 1 H, J = 2.4 5z (OCH$_2$CC*H*); δ = 2.00 ppm, s, 3 H (OCOC*H*$_3$); δ = 1.94 ppm, s, 3 H (OCOC*H*$_3$); δ = 1.88 ppm, s, 3 H (OCOC*H*$_3$); δ = 1.83 ppm, s, 3 H (OCOC*H*$_3$).

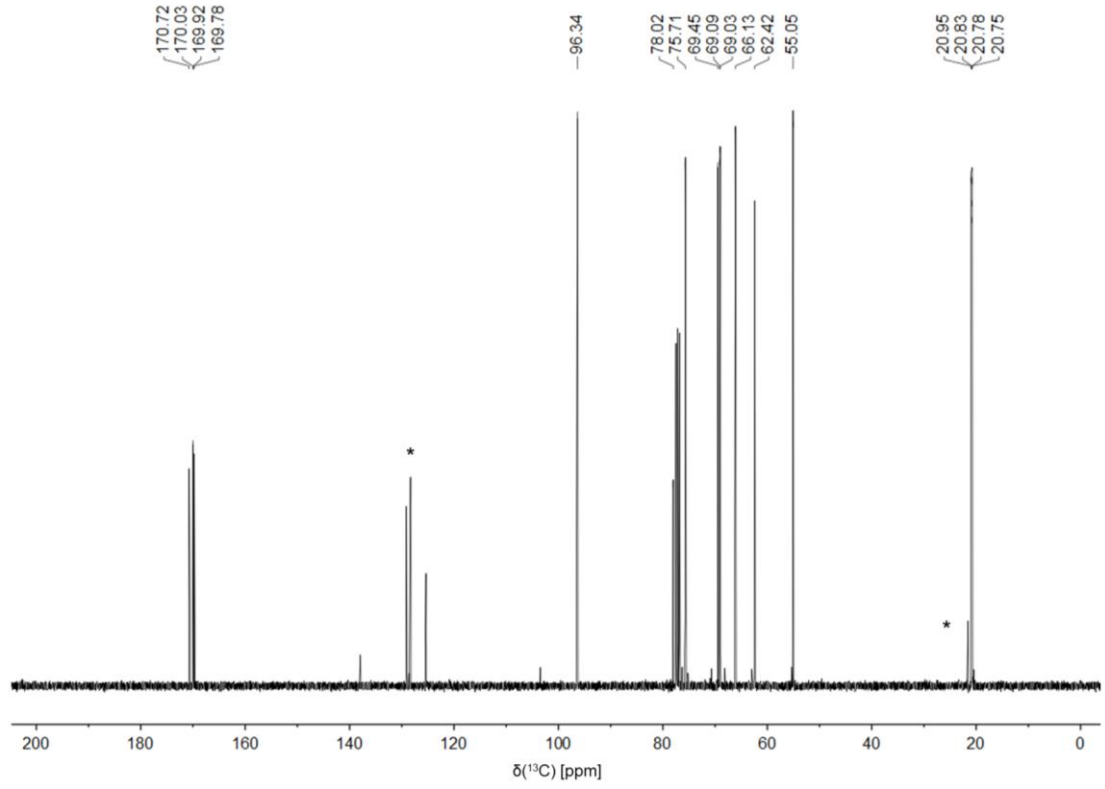
^{13}C NMR (100.6 MHz, CDCl3): δ = 170.7 ppm, 1 C (O*C*OCH$_3$); δ = 170.0 ppm, 1 C (O*C*OCH$_3$); δ = 169.9 ppm, 1 C (O*C*OCH$_3$); δ = 169.8 ppm, 1 C (O*C*OCH$_3$); δ = 95.5 ppm, 1 C (C1); δ = 96.3 ppm, 1 C (C1); δ = 78.0 ppm, 1 C (OCH$_2$*C*CH); δ = 75.7 ppm, 1 C (OCH$_2$C*C*H); δ = 69.5 ppm, 1 C (C2); δ = 69.1 ppm, 1 C (C5); δ = 69.0 ppm, 1 C (C3); δ = 66.1 ppm, 1 C (C4); δ = 62.4 ppm, 1 C (C6); δ = 55.1 ppm, 1 C (O*C*H$_2$CCH); δ = 20.9 ppm, 1 C (OCO*C*H$_3$); δ = 20.8 ppm, 2 C (three times OCO*C*H$_3$).

R_f = 0.38 with toluene:ethyl acetate (3:1).

ESI-MS for $C_{17}H_{22}O_{10}$: $m{\cdot}z^{-1}(M+Na^+)_{calc}$ = 409.1; $m{\cdot}z^{-1}(M+Na^+)_{obs}$ = 409.2; $m{\cdot}z^{-1}(M+NH_4^+)_{calc}$ = 404.2; $m{\cdot}z^{-1}$ $(M+NH_4^+)_{obs}$ = 404.2.



698

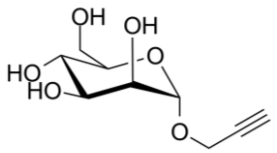


699

700 *Sample contained residual toluene. The yield was determined after removal of the solvent *in vacuo*.

701

Propargyl-α-D-mannopyranoside



5.0

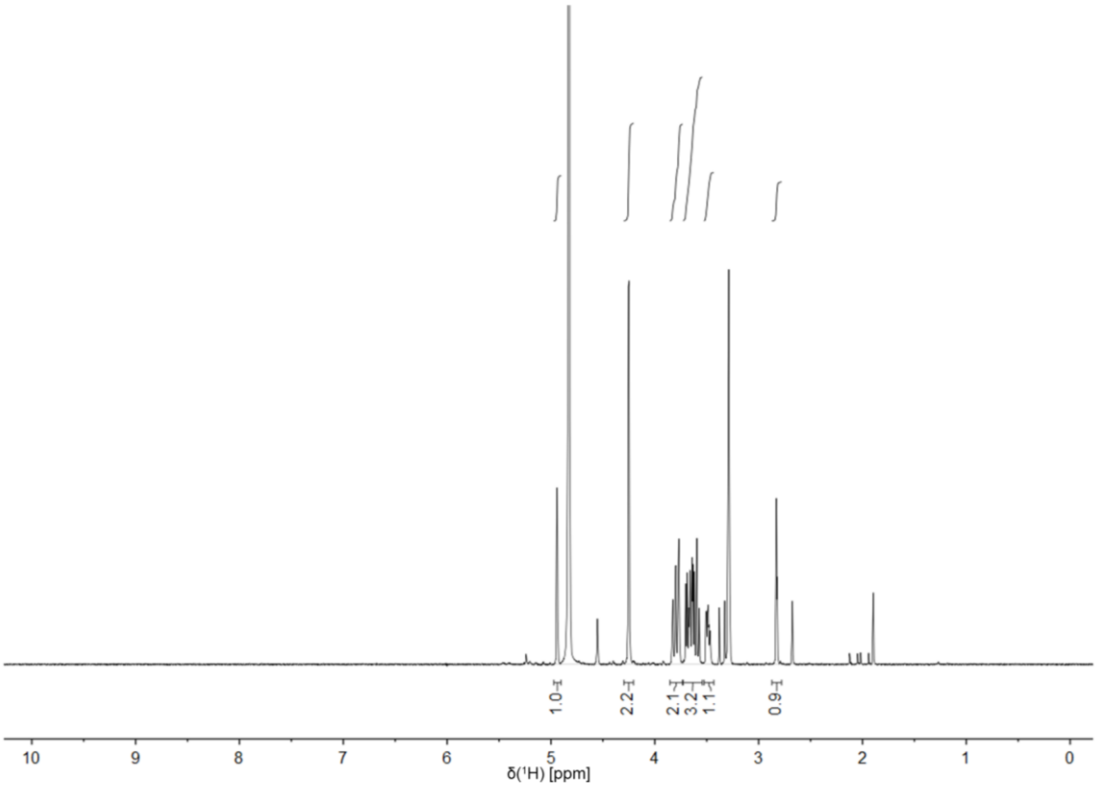
7 (188 mg, 487 µmol) was dissolved in EtOH containing 33% methylamine (13 ml) at room temperature. The mixture was stirred overnight and progress of the reaction was monitored by analytical TLC (20% MeOH in DCM). Solvents were evaporated *in vacuo* and the residue was purified via column chromatography (gradient: hexane, hexane:DCM (1:1), DCM, 1% MeOH in DCM, 5% MeOH in DCM and elution with 20% MeOH in DCM). Silica gel particles were removed by filtration in MeOH with a cellulose acetate membrane at a pore size 0.2 µm to yield **5.0** (91 mg, 417 µmol, 86%) as a white solid.

^{1}H NMR (400.0 MHz, MeOD): δ = 4.95 ppm, d, 1 H, J = 1.2 Hz (H1); δ = 4.26 ppm, d, 2 H, J = 2.6 Hz (OC*H*$_2$CCH); δ = 3.79 ppm, dd, 1 H, J = 2.2, 11.7 Hz (H6a); δ = 3.76 ppm, dd, 1 H, J = 1.6, 3.1 Hz (H2); δ = 3.70 ppm, m, 1 H (H6b); δ = 3.65 ppm, m, 1 H (H3); δ = 3.61 ppm, m, 1 H (H4); δ = 3.50 ppm, ddd, 1 H, J = 2.0, 5.8, 9.6 Hz (H5); δ = 2.85 ppm, t, 1 H, J = 2.4 Hz (OCH$_2$CC*H*).

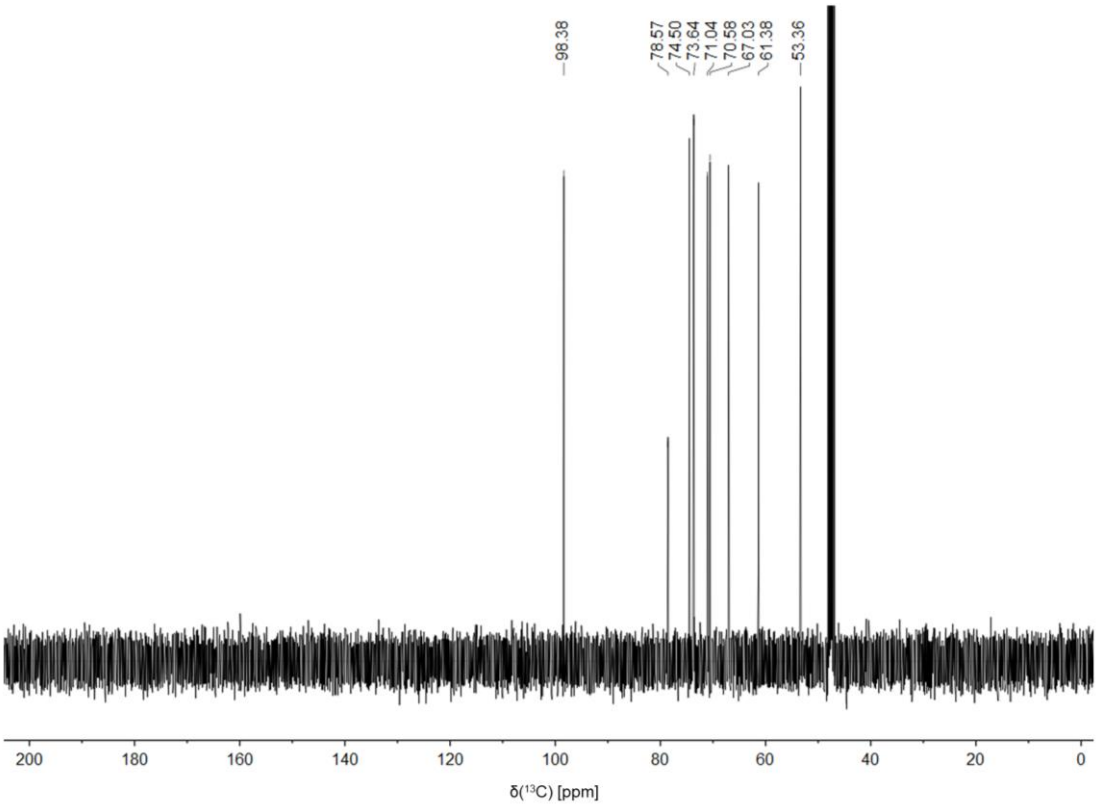
^{13}C NMR (100.6 MHz, MeOD): δ = 99.8 ppm, 1 C (C1); δ = 80.0 ppm, 1 C (OCH$_2$*C*CH); δ = 76.0 ppm, 1 C (OCH$_2$C*C*H); δ = 75.1 ppm, 1 C (C5); δ = 72.5 ppm, 1 C (C3); δ = 72.0 ppm, 1 C (C2); δ = 68.5 ppm, 1 C (C4); δ = 62.8 ppm, 1 C (C 6); δ = 54.8 ppm, 1 C (O*C*H$_2$CCH).

R_f = 0.36 with 20% MeOH in DCM.

HR ESI-MS for $C_9H_{14}O_6$: $m \cdot z^{-1}(M+Na^+)_{calc}$ = 241.069, $m \cdot z^{-1}(M+Na^+)_{obs}$ = 241.068.


1.0
2.2
2.1
3.2
1.1
0.9
10
9
8
7
6
5
4
3
2
1
0
δ(¹H) [ppm]

720


98.38
78.57
74.50
73.64
71.04
70.58
67.03
61.38
53.36
200
180
160
140
120
100
80
60
40
20
0
δ(¹³C) [ppm]

721

722

^{19}F R_2-filtered NMR

^{19}F R_2-filtered NMR experiments were conducted on a OneNMR 600 MHz spectrometer (Agilent). Spectra were processed in MestReNova and data analysis was performed with OriginPro (*19, 22*). Experiments utilizing the Langerin ECD were performed at a receptor concentration of 50 µM in 25 mM Tris with 10% D_2O, 150 mM NaCl and 5 mM $CaCl_2$ at pH 7.8 and 25° C. Experiments utilizing the Langerin CRD were performed at a receptor concentration of 50 µM in 25 mM HEPES with 10% D_2O, 150 mM NaCl and 5 mM $CaCl_2$ at pH 7.0 and 25°C. Trifluoroacetic acid served as an internal reference at a concentration of 50 or 100 µM. For each spectrum 128 scans were recorded in 3 mm sample tubes at sample volumes of 150 µl. Relaxation rates $R_{2,obs}$ were determined with the CPMG pulse sequence by fitting Equation 1 to integrals of the ^{19}F resonance of **5.1** (*23*). T represents the relaxation time and I_0 is the integral at a T value of 0 s. The relaxation delay d_1 was set to 2.0 s, the acquisition time t_{acq} was set to 0.8 s and the frequency of 180° pulses ν_{CPMG} was set to 500 Hz.

$$I = I_0 e^{-R_{2,obs}T}$$

Equation 1

The K_D and the $R_{2,b}$ value of the reporter molecule **5.1** were derived from Equation 2 by detection of ^{19}F NMR relaxation rates $R_{2,obs}$ in a two parameter fit (*24*, *25*). $R_{2,b}$ represents the relaxation rate in bound state of the ligand and p_b is the bound fraction of the ligand while $[L]_T$ and $[P]_T$ represent the concentrations of ligand and receptor, respectively. The relaxation rate of the free ligand $R_{2,f}$ was measured at 0.1 mM **5.1** in absence of the receptor. The EDTA control experiment was conducted at 12.5 mM **5.1**. To ensure the validity of Equation 2, the chemical exchange contribution $R_{2,ex}$ was estimated by ^{19}F NMR relaxation dispersion experiments at 0.1 mM **5.1**.

$$R_{2,obs} = R_{2,f} + (R_{2,b} - R_{2,f})p_b$$

with

$$p_b = \left(\frac{[P]_T + [L]_T + K_D - \sqrt{([P]_T + [L]_T + K_D)^2 - 4[P]_T[L]_T}}{2[L]_T} \right)$$

Equation 2

For the competitive binding experiments in Setup 1, binding of 0.1 mM **5.1** to the ECD was detected at five or more competitor concentrations $[I]_T$. Equation 3 served to derive $[P]_T$ and K_I values from $R_{2,obs}$ values in a two parameter fit (*25*). The pH value of stock solutions of **5.11** was adjusted to 7.8 prior to titration experiments using 1 M NaOH. In Setup 2, $[P]_T$ values were directly calculated from

the data point at 0.1 mM **5.1** and in absence of competitor via Equation 1. Subsequently, K_I values were estimated from Equation 2 in a one parameter fit. Data points were selected for the evaluation of the assay performance if the competitor concentrations fell within one order of magnitude of the K_I value determined in Setup 1. Deviations from Setup 1 were quantified via the calculation of ΔK_I values.

$$R_{2,obs} = R_{2,f} + \left(R_{2,b} - R_{2,f}\right)p_b$$

with

$$p_b = \frac{2cos\left(\frac{\theta}{3}\right)\sqrt{a^2 - 3b} - a}{3K_D + 2cos\left(\frac{\theta}{3}\right)\sqrt{a^2 - 3b} - a}$$

and

$$\theta = cos^{-1}\left(\frac{-2a^3 + 9ab - 27c}{2\sqrt{(a^2 - 3b)^3}}\right), \qquad a = K_D + K_I + [L]_T + [I]_T - [P]_T,$$

$$b = ([I]_T - [P]_T)K_D + ([L]_T - [P]_T)K_D + K_I K_D, \qquad c = -K_I K_D [P]_T$$

Equation 3

The fragment screening was conducted using the ECD in presence of 10% DMSO and 0.01% Tween-20. The influence of the additives on assay performance was evaluated via titration and screening experiments with Man. Additionally, the mean $\mu_{Reference}$ and standard deviation σ of R_2,obs values in absence of competitor were estimated from independent experiments (n = 9). Overall, 290 fragments (Key Organics) were randomly selected from our in-house library. These fragments were binned into mixtures of 5 or 6 and screened at concentration of 0.5 mM. A 3σ-threshold was utilized to define screening hits and estimated K_I values for Man and **8** were determined in Setup 2.

^{15}N HSQC NMR

^{15}N HSQC NMR experiments were conducted on a OneNMR 600 MHz spectrometer (Agilent). Spectra were processed in NMRPipe (*26*). Data analysis was performed in CCPN Analysis and OriginPro (*22, 27*). Experiments were conducted with Langerin CRD concentrations between 160 and 200 µM in 25 mM HEPES with 10% D_2O, 150 mM NaCl and 5 mM $CaCl_2$ at pH 7.0 and 25°C. DSS served as an internal reference at a concentration of 100 µM. Spectra were acquired with 128 increments and 8 scans per increment for 500 µl samples in 5 mm sample tubes and 32 scans per increments for 150 µl samples in 3 mm sample tubes. The relaxation delay d_1 was set to 0.15 s and the acquisition time t_{acq} was set to 1.35 s. The W5 Watergate pulse sequence was utilized for solvent suppression (*28*). CSPs for receptor resonances in the fast or fast-to-intermediate exchange regime observed upon titration with ligand were calculated via Equation 4 (*29*).

$$CSP = \sqrt{\frac{\delta(^{1}\mathrm{H})^2 + \left(0.15\delta(^{15}N)\right)^2}{2}}$$

Equation 4

Resonances that displayed CSP values higher than 0.04 ppm at the highest ligand concentration were selected for the determination of K_D values via Equation 5 in a global two parameter fit (*29*). CSP_{max} represents the CSP value observed upon saturation of the binding site.

$$CSP = CSP_{max} p_b$$

with

$$p_b = \left(\frac{[P]_T + [L]_T + K_D - \sqrt{([P]_T + [L]_T + K_D)^2 - 4[P]_T[L]_T}}{2[P]_T} \right)$$

Equation 5

The pH value of stock solutions of **5.11** was adjusted to 7.0 prior to titration experiments using 1 M NaOH.

^{1}H STD NMR

^{1}H STD NMR experiments were conducted on a OneNMR 600 MHz spectrometer (Agilent) and spectra were processed in MestReNova (*19, 30*). Experiments were conducted utilizing the Langerin ECD at a receptor concentration of 25 µM in 25 mM Tris-d_{11} with 100% D_2O, 150 mM NaCl and 5 mM $CaCl_2$ at pH 7.8 and 25° C. The experiment was repeated in absence of receptor to exclude STD effects due to direct saturation of fragments. Residual H_2O served as an internal reference. For each spectrum 512 scans were recorded in 5 mm sample tubes at sample volumes of 500 µl. Saturation was implemented via a train of Gauss pulses at a saturation time t_{sat} of 4.0 s. The on-resonance irradiation frequency ν_{sat} was set to 0.0 ppm and the off-resonance irradiation frequency ν_{ref} was set to 80.0 ppm. The relaxation delay d_1 was set to 0.0 s and the acquisition time t_{acq} was set to 2.0 s. The DPFGSE pulse sequence was utilized for solvent suppression (*31*). Receptor resonances were suppressed via a $T_{1,rho}$ filter at a relaxation time τ of 35 ms. Resonances of the analyzed fragment mixture were assigned by comparison to previously acquired ^{1}H NMR spectra of the individual fragments.

III. References

1. Duus, J., Gotfredsen, C. H., and Bock, K. (2000) Carbohydrate structural determination by NMR spectroscopy: modern methods and limitations. *Chem. Rev. 100*, 4589-4614.

2. Feinberg, H., Taylor, M. E., Razi, N., McBride, R., Knirel, Y. A., Graham, S. A., Drickamer, K., and Weis, W. I. (2011) Structural basis for langerin recognition of diverse pathogen and mammalian glycans through a single binding site. *J. Mol. Biol. 405*, 1027-1039.

3. Mannhold, R., Poda, G. I., Ostermann, C., and Tetko, I. V. (2009) Calculation of molecular lipophilicity: State-of-the-art and comparison of log P methods on more than 96,000 compounds. *J. Pharm. Sci. 98*, 861-893.

4. Chen, I. J., and Hubbard, R. E. (2009) Lessons for fragment library design: analysis of output from multiple screening campaigns. *J. Comput. Aided Mol. Des. 23*, 603-620.

5. Keseru, G. M., Erlanson, D. A., Ferenczy, G. G., Hann, M. M., Murray, C. W., and Pickett, S. D. (2016) Design Principles for Fragment Libraries: Maximizing the Value of Learnings from Pharma Fragment-Based Drug Discovery (FBDD) Programs for Use in Academia. *J. Med. Chem. Online*.

6. Chemical Computing Group (2013) Molecular Operating Environment, *2013.08*.

7. Case, D. A., Darden, T. A., Cheatham, T. E., Simmerling, C. L., Wang, J., Duke, R. E., Luo, R., Crowley, M., R.C.Walker, Zhang, W., Merz, K. M., B.Wang, Hayik, S., Roitberg, A., Seabra, G., Kolossváry, I., K.F.Wong, Paesani, F., Vanicek, J., X.Wu, Brozell, S. R., Steinbrecher, T., Gohlke, H., Yang, L., Tan, C., Mongan, J., Hornak, V., Cui, G., Mathews, D. H., Seetin, M. G., Sagui, C., Babin, V., and P.A. Kollman (2008) AMBER, *10*.

8. Gerber, P. R., and Müller, K. (1995) MAB, a generally applicable molecular force field for structure modelling in medicinal chemistry. *J. Comput. Aided Mol. Des. 9*, 251-268.

9. Halgren, T. A. (1996) Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. *J. Comput. Chem. 17*, 490-519.

10. Berthold, M. R., Cebron, N., Dill, F., Gabriel, T. R., Koetter, T., Meinl, T., Ohl, P., Sieb, C., Thiel, K., and Wiswedel, B. (2007) KNIME: The Konstanz Information Miner, in *Studies in Classification, Data Analysis, and Knowledge Organization*, Springer.

11. Feinberg, H., Rowntree, T. J., Tan, S. L., Drickamer, K., Weis, W. I., and Taylor, M. E. (2013) Common polymorphisms in human langerin change specificity for glycan ligands. *J. Biol. Chem. 288*, 36762-36771.

12. Chen, V. B., Arendall, W. B., 3rd, Headd, J. J., Keedy, D. A., Immormino, R. M., Kapral, G. J., Murray, L. W., Richardson, J. S., and Richardson, D. C. (2010) MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr. Sect. D-Biol. Crystallogr. 66*, 12-21.

13. Labute, P. (2010) LowModeMD - implicit low-mode velocity filtering applied to conformational search of macrocycles and protein loops. *J. Chem. Inf. Model. 50*, 792-800.

14. Oprea, T. I. (2000) Property distribution of drug-related chemical databases. *J. Comput. Aided Mo.l Des. 14*, 251-264.

15. ChemAxon (2014) Marvin, *14.7.7.0*.

16. Stambach, N. S., and Taylor, M. E. (2003) Characterization of carbohydrate recognition by langerin, a C-type lectin of Langerhans cells. *Glycobiology 13*, 401-410.

17. Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M. R., Appel, R. D., and Bairoch, A. (2005) Protein identification and analysis tools on the ExPASy server, in *The Proteomics Protocols Handbook* (Walker, J. M., Ed.), 571-607, Humana Press.

18. Duus, J., Gotfredsen, C. H., and Bock, K. (2000) Carbohydrate structural determination by NMR spectroscopy: modern methods and limitations. *Chem. Rev. 100*, 4589-4614.

19. Mestrelab Research (2015) MestreNova, *10.0.2*.

20. Veselý, J., Ledvina, M., Jindrich, J., Šaman, D., and Trnka, T. (2003) Improved synthesis of 1,2-trans-acetates and 1,2-trans ethyl 1-thioglycosides derived from 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-d-hexopyranosides. *Collect. Czech. Chem. Comm. 68*, 1264-1274.

21. Hasegawa, T., Numata, M., Okumura, S., Kimura, T., Sakurai, K., and Shinkai, S. (2007) Carbohydrate-appended curdlans as a new family of glycoclusters with binding properties both for a polynucleotide and lectins. *Org. Biomol. Chem. 5*, 2404-2412.

22. OriginLab (2015) OriginPro, *9.1*.

23. Carr H. Y., and M., P. E. (1954) Effects of diffusion on free precession in nuclear magnetic resonance experiments. *Phys. Rev. 94*.

24. Dalvit, C. (2007) Ligand- and substrate-based 19F NMR screening: Principles and applications to drug discovery. *Prog. Nucl. Mag. Res. Sp. 51*, 243-271.

25. Roehrl, M. H., Wang, J. Y., and Wagner, G. (2004) A general framework for development and data analysis of competitive high-throughput screens for small-molecule inhibitors of protein-protein interactions by fluorescence polarization. *Biochemistry 43*, 16056-16066.

26. Delaglio, F., Grzesiek, S., Vuister, G. W., Zhu, G., Pfeifer, J., and Bax, A. (1995) NMRPipe: A multidimensional spectral processing system based on UNIX pipes. *J. Biomol. NMR 6*, 277-293.

27. Vranken, W. F., Boucher, W., Stevens, T. J., Fogh, R. H., Pajon, A., Llinas, M., Ulrich, E. L., Markley, J. L., Ionides, J., and Laue, E. D. (2005) The CCPN data model for NMR spectroscopy: development of a software pipeline. *Proteins 59*, 687-696.

28. Liu, M., Mao, X.-a., Ye, C., Huang, H., Nicholson, J. K., and Lindon, J. C. (1998) Improved WATERGATE pulse sequences for solvent suppression in NMR spectroscopy. *J. Magn. Reson. 132*, 125-129.

29. Williamson, M. P. (2013) Using chemical shift perturbation to characterise ligand binding. *Prog. Nucl. Magn. Reson. Spectrosc. 73*, 1-16.

30. Mayer, M., and Meyer, B. (1999) Characterization of ligand binding by saturation transfer difference NMR spectroscopy. *Angew. Chem. Int. Ed. 38*, 1784-1788.

31. Hwang, T. L., and Shaka, A. J. (1995) Water Suppression That Works - Excitation Sculpting Using Arbitrary Wave-Forms and Pulsed-Field Gradients. *J. Magn. Reson. Ser. A 112*, 275-279.