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Synthesis of Cluster Mannosides Carrying a Photolabile Diazirine Group

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We are investigating the mechanisms underlying the carbohydrate-specific adhesion of bacteria such as *Escherichia coli* to the glycocalyx of their potential host cells. *E. coli* possess protein appendages, which are called type 1 fimbriae. Part of type 1 fimbriae is a protein named FimH, which is a mannose-specific lectin. We wish to use photoaffinity labeling to elucidate mannose binding sites on FimH. Thus we report the synthesis of di- and trivalent cluster mannosides, which carry a photolabile diazirine group. The diazirine group was introduced by a convergent approach using thiourea bridging (products **6**, **13**, **17**, and **27**) or in a divergent synthesis leading to the divalent cluster mannoside **31**.
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Introduction

Photoaffinity labeling is a powerful method of biological chemistry[1] to study receptor binding sites, such as the active site of an enzyme, e.g., or the carbohydrate recognition domain (CRD) of a lectin, respectively. It enables direct probing of a target receptor through a covalent bond, which is photochemically introduced between the ligand and the specific protein.

Among several photoreactive groups commonly used in photoaffinity labeling,[2] diazirines[3] have often been shown to be advantageous over others.[4] Upon irradiation diazirines produce a reactive carbene after the light-induced loss of nitrogen. This photogenerated intermediate is very reactive and can insert into single bonds allowing the characterization of carbohydrate-binding proteins, for instance.[5]

In the course of our work on the investigation of carbohydrate binding to the bacterial lectin FimH[6] we have envisaged photoaffinity labeling of FimH using suitable carbohydrate derivatives which carry a photolabile diazirine group. The bacterial lectin FimH is part of the so-called type 1 fimbriae, which are protein appendages on the surface of *Escherichia coli* and are utilized for carbohydrate-specific adhesion of the bacteria to the host cell glycocalyx. X-ray studies have revealed that the lectin domain of type 1 fimbriae, the protein FimH, possesses a CRD at its tip, which can accommodate one α-mannoside residue.[7] However, theoretical studies[8] as well as testing results with multivalent mannosyl clusters[9] have suggested, that there might be additional carbohydrate binding domains on FimH.

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To verifiy this hypothesis using the photoaffinity labeling technology, we have defined di- and trivalent cluster mannosides as our target molecules, which carry a photolabile diazirine group. Ideally, incubation of such photolabile cluster mannosides with FimH leads to covalently linked glycocluster-FimH conjugates, giving information about carbohydrate binding sites on the protein. Thus, the synthesis of divalent and trivalent photolabeled cluster mannosides is described in this contribution.

Results and Discussion

We have recently reported a convergent approach for the synthesis of diazirine-labeled carbohydrate derivatives, which is based on thiourea bridging of an amino-functionalized diazirine derivative (**4**, Scheme 1) and isothiocyanato-functionalized sugars.[10] As thiourea-bridged glycoclusters have been shown to be highly interesting probes for biological studies,[11] we have also envisaged this approach for the preparation of photolabeled glycoclusters. Earlier on, thiourea-bridging has led to photolabile glycoclusters carrying three diazirine groups utilizing the diisothiocyanato-functionalized mannose derivative **1**.[10] However, we figured that glycoclusters carrying more than one diazirine group are not ideally suited as photoaffinity probes for FimH because irradation would always activate all photolabile groups present in the molecule and eventually lead to a puzzling armada of products. Therefore, we tried to synthesise mannose clusters with a single diazirine group and have attempted to use an anlogous approach as reported earlier.[10] Thus, the isothiocyanato-functionalized mannosyl isothiocyanate **1** was treated with tris(2-aminoethyl)-amine (**2**) to obtain the glycocluster **3** in 70% yield (Scheme 1). Then, we have evaluated the feasibility of mono-functionalization of **3** with the diazirine **4**, however, only the synthesis of the tri-functionalized **5** was satisfac-

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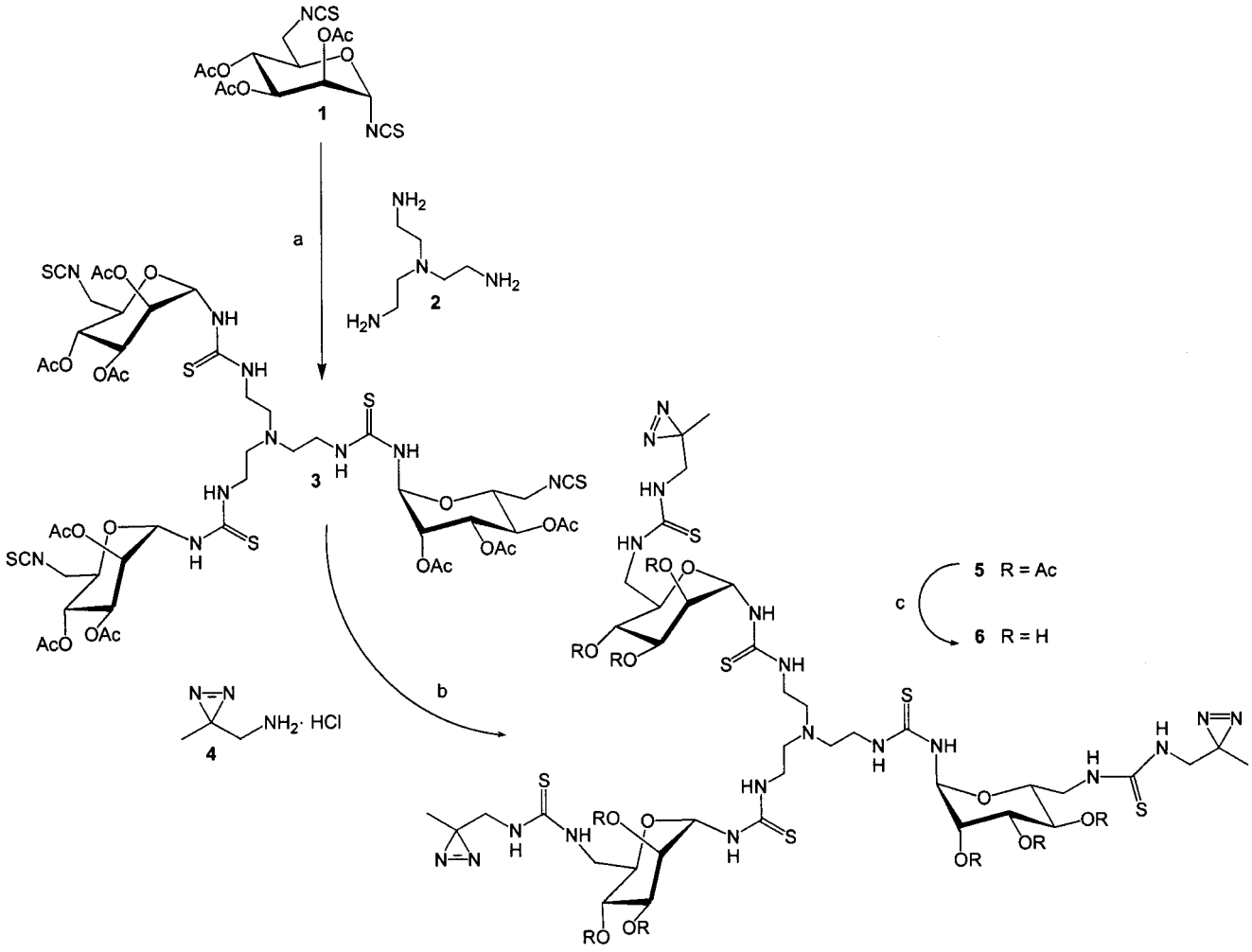

Scheme 1. a) Dichloromethane, 0 °C, 2 h, 70%; b) dichloromethane, DIPEA, room temp., 2 h, 52%; c) 1 M NaOMe in MeOH, 0 °C, 1 h, 69%.

tory. While deprotection of **5** according to Zemplén[12] leading to the water-soluble unprotected glycocluster **6** was convenient, this was not the type of product we had targeted. To achieve the preparation of cluster mannosides with only one diazirine group present in the molecule, we have employed 2-hydroxymethyl-1,3-propandiol (Scheme 2) as the core molecule instead of the branched trisamine **2**.

A straightforward reaction sequence starting with 2-hydroxymethyl-1,3-propandiol led to the isopropylidene-protected azide **7** via isopropylidination, tosylation of the remaining hydroxy group and nucleophilic substitution by sodium azide in one pot (Scheme 2). The azide **7** was purified and subjected to cleavage of the dioxane ring to yield the diol **8**, which served as mannosyl acceptor in the following glycosylation reaction. Mannosylation was carried out according to the trichloroacetimidate method[13] using the benzoyl-protected mannosyl donor **9**[14] and TMS-triflate as the Lewis acid. Almost one equivalent of the glycosyl donor per hydroxy group was enough to deliver the target compound **10** in 75% yield.

The azido group at the focal point of the divalent mannoside **10** offers several options for further derivatization. One possibility is to turn the azide group into an NCS function in a Staudinger-type reaction by using triethyl phosphite and carbon disulfide[15] to yield the isothiocyanate **11**. The latter was allowed to react with the hydrochloride **4**, which had to be converted into the respective amine by treatment with DIPEA, to finally furnish the targeted photolabeled bis-mannoside **12**. Because the Staudinger reaction as well as thiourea bridging in the following step gave lower yields than usual, we assume that the reactivity at the focal point of **10** and **11** is diminished, possibly due to steric hindrance. Deprotection of **12** led to the OH-free compound **13** which can be regarded as a photolabile disaccharide mimetic.[16]

For a second synthetic pathway we planned to reduce the azide **10** to the respective amine **14**. Surprisingly this was not possible although several different reaction conditions were applied. Finally, Staudinger reaction using triphenyl phosphane provided the desired product **14** in moderate yield, which was used without further purification (Scheme 2). The reaction with the NCS-functionalized mannose derivative **15**[10] carrying a diazirine group led to the trivalent mannosyl cluster **16**, which could be de-acyl-

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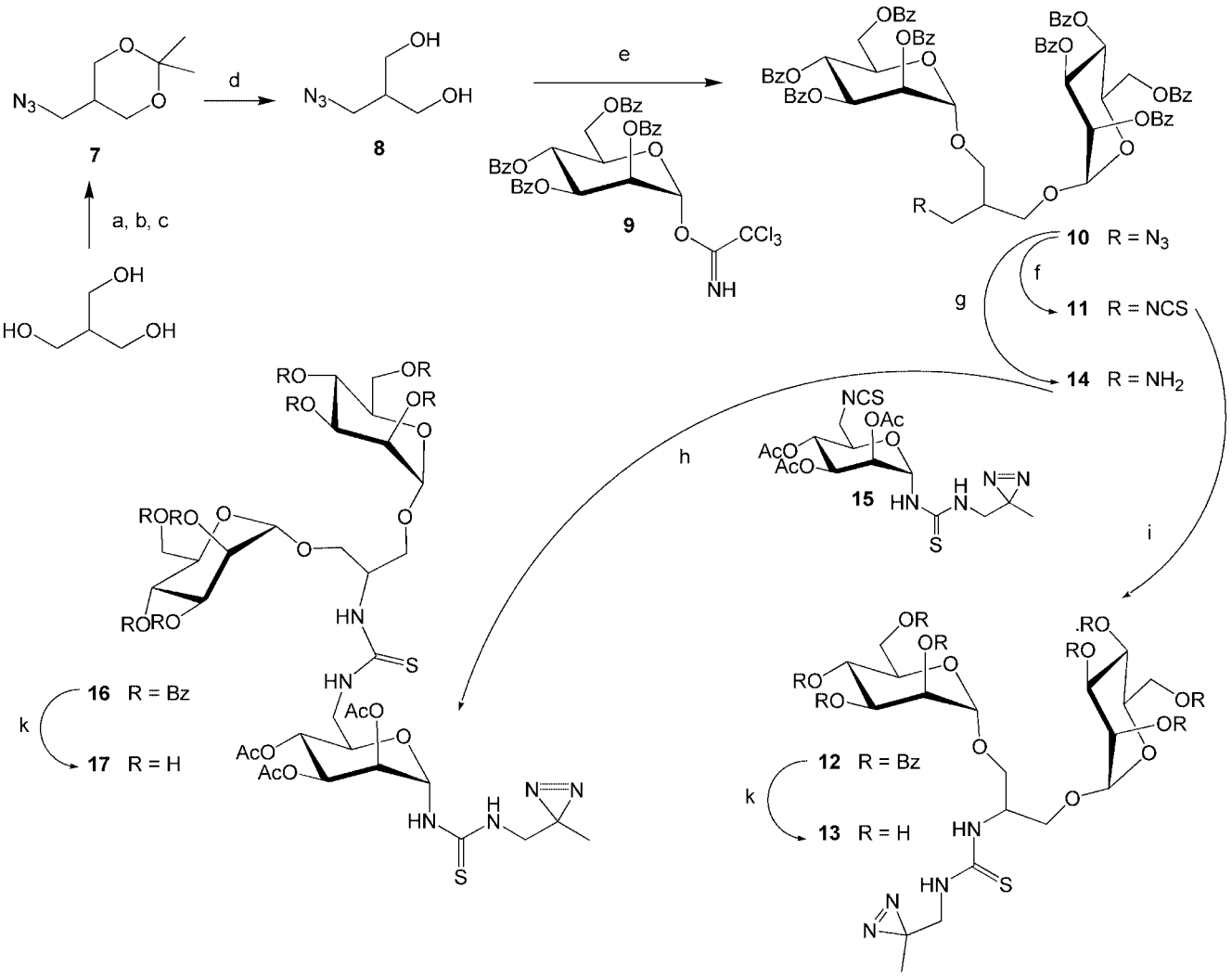

Scheme 2. a) DMP, acetone, TsOH, THF, room temp., 1.5 h; then b) TsCl, pyridine, room temp., 12 h; then c) NaN_3, TBABr, toluene, 60 °C, 24 h, 41% over three steps. d) HCl, MeOH, room temp., 1 h, quant. e) TMS-OTf, dichloromethane, Ar, 0 °C → room temp., 1 d, 75%. f) CS_2, $P(OEt)_3$, toluene, 80 °C, 8 h, 56%. g) PPh_3, THF, Ar, 0 °C → room temp., 1 h; then h) H_2O, 23% over two steps. i) **4**, DIPEA, dichloromethane, room temp., 2 h, 48%. k) 1 M NaOMe in MeOH/THF, room temp., 1 h, 65% for **13**; 63% for **17**.

ated according to Zemplén to yield **17**. Both unprotected glycoclusters **13** and **17** carry differently positioned single diazirine groups. This is a favorable variation with regard to the eventual biological application of these molecules, as the optimal position of the photoactive group within the carbohydrate probe is not known and can hardly be anticipated.

A similar approach as applied for the synthesis of **13** was then used to prepare a branched trivalent cluster mannoside based on a pentaerythritol derivative as the core molecule, carrying the diazirine group on the scaffold (Scheme 3). The synthesis started with triallyl pentaerythritol (**18**) which was easily tosylated to **19**. The tosylate was subjected to reductive ozonization to yield the tris(hydroxyethyl) derivative **20**. To facilitate purification, this product was acetylated to **21** prior to the following nucleophilic substitution step with sodium azide, leading to the azido-functionalized, *O*-acetyl-protected triol **22**, which could readily be purified. After Zemplén deprotection the core molecule **23** was ready for glycosylation. Again, only a minimum excess of the mannosyl donor **9** was necessary to deliver the trivalent cluster mannoside **24** in good yield.

The azido-functionalized cluster **24** could be converted into its isothiocyanato-functionalized analogue **25**, as described for **10** and thiourea bridging with **4** delivered the photolabeled trivalent cluster mannoside **26**. Unfortunately, the yield of the following deprotection reaction to give **27** did not exceed 55%.

Finally we wanted to find out if it is possible to bind a diazirine function to the focal point of glycodendrons of the glycoglycerol type, which we have recently introduced.[17] Therefore, we used the alkene **28**, carrying two isopropylidene-protected α-mannosyl residues as the starting material. Ozonolysis was successful and work-up with triphenylphosphane yielded the cluster mannoside **29** with a carbonyl function at its focal point. The ketone **29** was subjected to the usual reaction sequence for the conversion into a diazirine ring.[18] Reaction with hydroxylamine-*O*-

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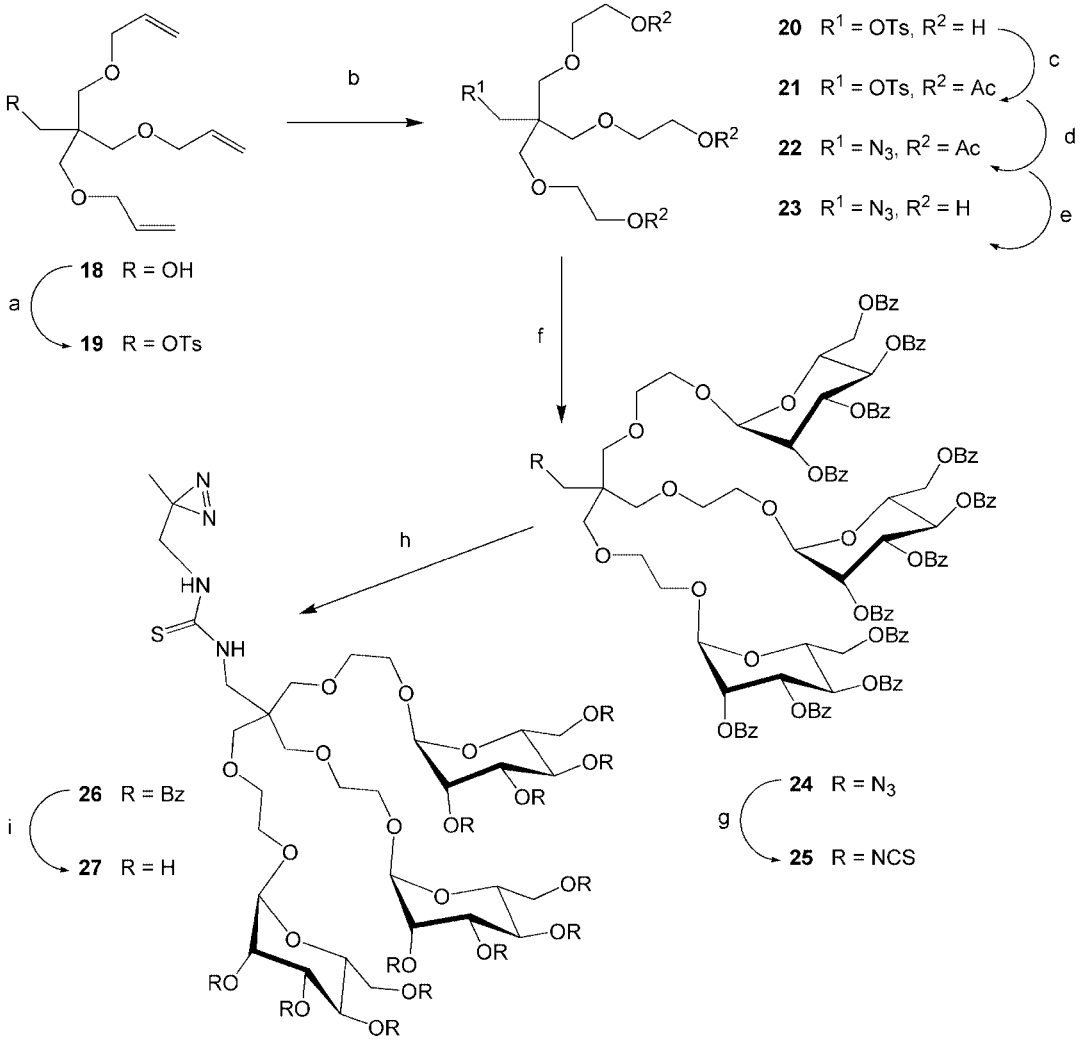

Scheme 3. a) TsCl, pyridine, room temp., 16 h, 81%. b) O_3, MeOH, –78 °C, 0.5 h; then $NaBH_4$, 46%. c) Ac_2O, pyridine, room temp., 16 h, 84%. d) NaN_3, TBABr, DMF, 80 °C, 6 d, 93%. e) 1 M NaOMe in MeOH, room temp., 0.5 h, 91%. f) **9**, TMS-OTf, dichloromethane, Ar, 0 °C → room temp., 1 d, 67%. g) CS_2, $P(OEt)_3$, toluene, 80 °C, 8 h, 83%. h) **4**, DIPEA, dichloromethane, 30 °C, 8 h, 80%. i) 1 M NaOMe in MeOH/THF, room temp., 1 h, 55%.

sulfonic acid in liquid ammonia, followed by oxidation of the intermediate diaziridine with iodine provided the photolabeled divalent cluster mannoside **30** in acceptable yield. Interestingly, a byproduct was formed and isolated in 24% yield, which was identified as the dimethoxy acetale analogue of ketone **29** (Scheme 4). Possibly the ammonium ion, which is formed after the addition of hydroxylamine-*O*-sulfonic acid under the almost water-free reaction conditions is acidic enough to catalyze the side reaction leading to the observed byproduct.

The acid-catalyzed deprotection of **30** was a high yielding reaction leading to the diazirine-labeled cluster **31** with intact glycosidic bonds.

A selection of the prepared diazirine-labeled saccharides was irradiated at wavelengths of ≥ 320 nm. Prior to irradiation the UV spectra were recorded and the decrease of the absorption maximum λ_{max} of the individual compound was observed during irradiation (Table 1). Irradition was carried out in MeOH or water and the respective insertion products could be detected in the ESI-MS. However, when the herein reported cluster mannosides were irradiated in the presence of more complex compounds such as a model peptide, complex mixtures were obtained, also including unsaturated products due to undesired H-abstraction in the intermediate carbene. Additional irradiation studies with model peptides as well as FimH are currently under investigation in our laboratory.

Table 1. UV spectroscopic data of selected diazirines.

	Solvent	Conc. [mM]	λ_{max} [nm]	λ_{max} disappearing after [min][a]
6	MeOH	0.5	342.2	30
13	H_2O	0.3	341.1	60
17	H_2O	0.3	342.2	60
27	H_2O	0.2	333.3	decomposition

[a] After irradiation at $\lambda \geq 320$ nm; during irradiation UV spectra were recorded in 5 minutes intervals.

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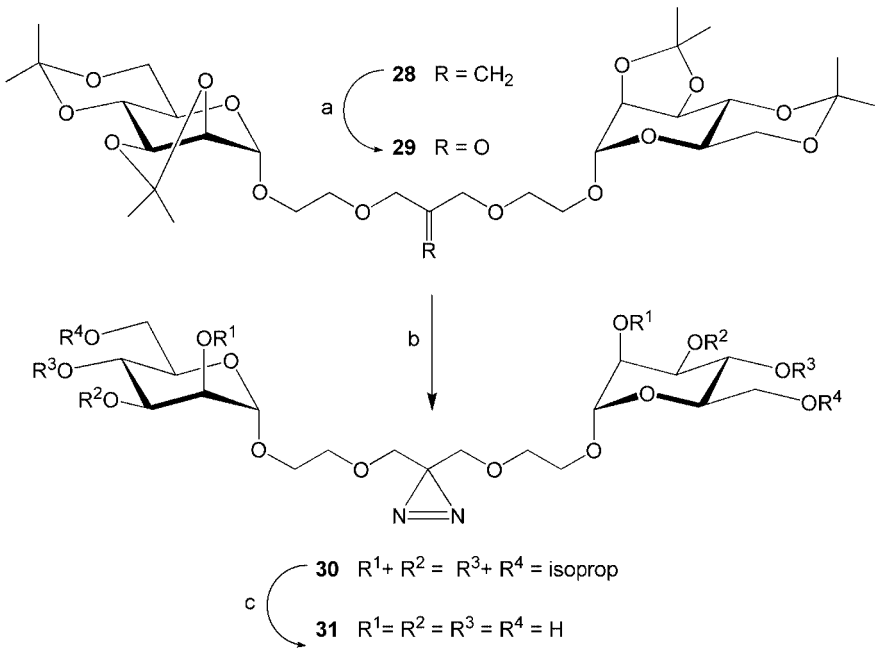

Scheme 4. a) O_3, MeOH/dichloromethane, –78 °C, 20 min; then PPh_3, 92%. b) NH_3, H_2NOSO_3H, MeOH/dichloromethane, –50 °C → –20 °C, 16 h; then 10% I_2/MeOH, TEA, 0 °C, 49%. c) TFA /H_2O, room temp., 10 min, 93%.

Conclusion

The reaction of photolabeled mannosyl clusters is feasible according to a convergent as well as a divergent synthetic approach. Strategies from various areas of organic chemistry such as glycodendron-like growth, thiourea-bridging and glycosidation can be favorably combined to furnish molecules in which the photolabile group is differently positioned. This is advantageous as the location of the photolabel in the probe, which is ideal for photoaffinity labeling of the protein under investigation, -FimH-, is not known and can hardly be predicted. A series of structurally varied, unprotected diazirine-labeled mannosyl clusters were obtained by the described method. These compounds lose nitrogen after 30 to 60 minutes upon irradiation at $\lambda \geq 320$ nm and will be evaluated for the investigation of the FimH protein as well as other mannose-specific lectins.

Experimental Section

General Remarks: Optical rotations were measured with a Perkin–Elmer polarimeter (20 °C, 589 nm, length of cuvette: 1 dm). Reactions were monitored by TLC on silica gel GF_{254} (Merck) with detection under UV and by charring with 10% sulfuric acid in ethanol and subsequent heating. Flash column chromatography was performed on silica gel 60 (40–63 μm, Merck). NMR spectra were recorded on Bruker AMX 400 or Bruker DRX 500 instruments. Chemical shifts are relative to TMS or the solvent peaks of $CDCl_3$ (δ = 7.24 ppm ^{1}H, 77.0 ppm ^{13}C) or [D_6]acetone (δ = 2.04 ppm ^{1}H, 29.3 ppm ^{13}C). Where necessary, assignments were based on COSY and HSQC experiments. Assignments indexed with an asterisk (*) are interchangable. IR spectra were taken with a Perkin–Elmer FT-IR Paragon 1000 (KBr). MALDI-TOF mass spectra were measured with a Bruker Biflex III with 19 kV acceleration voltage. DHB (c = 10 μg/μL in 40% acetonitrile/water, 0.1% TFA) was used as the matrix. Ionisation was effected with a nitrogen laser at 337 nm. ESI mass spectra were measured with an Applied Biosystems Mariner ESI-TOF 5280.

Tris{2-[3′-(2′′,3′′,4′′-tri-*O*-acetyl-6′′-deoxy-6′′-isothiocyanato-α-D-mannopyranosyl)thioureido]ethyl}amine (3): The diisothiocyanate **1** (155 mg, 0.40 mmol) was dissolved in dry dichloromethane (2 mL) and cooled to 0 °C. A solution of tris(2-aminoethyl)amine (**2**, 19.6 μL, 19.3 mg, 0.132 mmol, 0.33 equiv.) in dry dichloromethane (500 μL) was added in two subsequent portions after 30 minutes intervals. After 1 h at 0 °C, stirring was continued for 1 h at room temp., then the solvent was removed in vacuo and the residual syrup purified by flash chromatography (cyclohexane/ethyl acetate, 9:1). The title compound was obtained as an amorphous powder (123 mg, 94 μmol, 70%). R_f = 0.21 (ethyl acetate). $[\alpha]_D$ = +28.3 (c = 1.0 in $CHCl_3$). ^{1}H NMR (500 MHz, [D_6]acetone): δ = 8.14 (br. s, 3 H, 3 NH^{man}), 7.53 (br. s, 3 H, 3 $NHCH_2$), 6.18 (br. s, 3 H, 3 H-1), 5.51–5.43 (m, 3 H, 3 H-3), 5.36–5.26 (m, 6 H, 3 H-2, 3 H-4), 4.04–3.95 (m, 3 H, 3 H-5), 3.89 (dd, 3 H, 3 H-6′), 3.84–3.73 (m, 6 H, 3 H-6, 3 NHC*H*H), 3.72–3.60 (m, 3 H, 3 NHCH*H*), 2.84 [br. t, 6 H, 3 $N(CH_2)_3$], 2.16, 2.07, 1.99 [each s, each 9 H, 9 $C(O)CH_3$] ppm; $^3J_{2,3}$ = 2.6, $^3J_{3,4}$ = 9.4, $^3J_{5,6}$ = 3.9, $^3J_{5,6'}$ = 3.3, $^2J_{6,6'}$ = 15.6 Hz. ^{13}C NMR (125.75 MHz, [D_6]acetone): δ = 184.26 (3 C=S), 170.25, 170.20, 170.18 (9 C=O), 132.59 (3 NCS), 80.75 (3 C-1), 70.58 (3 C-5), 70.02 (3 C-2), 69.58 (3 C-3), 67.27 (3 C-4), 53.47 [3 $N(CH_2)_3$], 46.40 (3 C-6), 43.94 (3 $NHCH_2$), 20.80, 20.67, 20.59 [9 $C(O)CH_3$] ppm. MALDI-TOF MS (DHB): m/z = 1310.9 $[M]^+$ (calcd. m/z = 1310.27) for $C_{48}H_{66}N_{10}O_{21}S_6$.

Tris{2-[3′-[2′′,3′′,4′′-tri-*O*-acetyl-6′′-deoxy-6′′-(2′′′-azipropylthioureido)-α-D-mannopyranosyl]thioureido]ethyl}amine (5): The isothiocyanato-functionalized glycocluster **3** (110 mg, 85 μmol) was dissolved in dry dichloromethane (1 mL) and a solution of the diazirine **4** (31 mg, 0.25 mmol) in dry dichloromethane (500 μL) and DIPEA (50 μL) was added dropwise. The reaction mixture was stirred for 2 h at room temp., then the solvent was removed in vacuo. Flash chromatography (ethyl acetate/cyclohexane, 3:1) of the residue yielded the glycocluster **5** (70 mg, 44 μmol, 52%) as a

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white powder. R_f = 0.79 (ethyl acetate). $[\alpha]_D$ = +82.8 (c = 0.9 in $CHCl_3$). ^{1}H NMR (500 MHz, [D$_6$]acetone): δ = 8.39 (br. s, 3 H, 3 NH^{man}), 7.53, 7.39, 6.91, (each br. s, each 3 H, 9 NH), 6.08 (br. s, 3 H, 3 H-1), 5.40 (br. d, 3 H, 3 H-3), 5.31 (t~dd, 3 H, 3 H-2), 5.08 (t, 3 H, 3 H-4), 4.31–4.17 (br. m, 3 H, 3 H-6′), 4.11–4.02 (br. m, 3 H, 3 H-5), 3.93–3.80 [br. m, 3 H, 3 C*H*HC(NN)], 3.79–3.59 (br. m, 3 H, 3 H-6), 3.54–3.34 [m, 9 H, 3 NH–CH_2CH_2N, 3 CH*H*C(NN)], 2.84–2.79 (br. m, 3 H, 3 NC*H*H), 2.65–2.58 (br. m, 3 H, 3 NCH*H*), 2.16, 2.11, 2.08 [each s, each 9 H, 9 C(O)CH_3], 1.07 (s, 9 H, 3 CH_3) ppm; $^3J_{2,3}$ = 3.7, $^3J_{3,4}$ = 7.1, $^3J_{4,5}$ = 7.1 Hz. ^{13}C NMR (125.75 MHz, [D$_6$]acetone): δ = 185.07, 184.01 (6 C=S), 170.31, 170.25, 170.16 (9 C=O), 78.73 (3 C-1), 73.78 (3 C-5), 69.37 (3 C-2), 68.96 (3 C-4), 68.64 (3 C-3), 52.59 (3 NCH_2), 47.67 [3 CH_2C(NN)], 45.14 (3 C-6), 43.36 (3 NCH_2CH_2), 26.72 [3 C(NN)], 20.93, 20.76, 20.62 [9 C(O)CH_3], 18.30 (3 CH_3) ppm. ESI MS: m/z = 1566.4890 [M + H]$^+$ (calcd. m/z = 1566.4721) for $C_{57}H_{88}N_{19}O_{21}S_6$ m/z = 1588.5011; [M + Na]$^+$ (calcd. m/z = 1588.4540) for $C_{57}H_{87}N_{19}NaO_{21}S_6$.

Tris{2-[3′[6′′-deoxy-6′′-(2′′′-azipropylthioureido)-α-D-mannopyranosyl]thioureido]ethyl}amine (6): The acetylated glycocluster **5** (68 mg, 43 μmol) was dissolved in dry MeOH (500 μL), a solution in sodium methoxide (1 M in MeOH, 39 μL, 0.1 equiv. per OH) was added and the reaction mixture was stirred at 0 °C for 1 h. The solution was neutralized by addition of ion-exchange resin (Amberlite IR 120), filtered, washed with MeOH and then the solvent was removed in vacuo. The residue was purified by HPLC (Merck RP-8, 7 μm; MeCN/H_2O, 40:60). The purified fractions were lyophilized to yield the glycocluster **6** as a colorless amorphous solid (36 mg, 30 μmol, 69%). R_f = 0.21 (MeOH/ethyl acetate, 1:1). $[\alpha]_D$ = –19.8 (c = 0.88 in MeOH). ^{1}H NMR (500 MHz, [D$_6$]acetone): δ = 5.82, 5.64 (br. s, 3 H, 3 H-1), 4.06–3.50 [m, 30 H, 3 H-2, 3 H-3, 3 H-4, 3 H-5, 3 H-6, 3 H-6′, 3 NCH_2CH_2, 3 CH_2C(NN)], 2.87–2.74 (br. m, 6 H, 3 NCH_2), 1.11, 1.10, 1.09 (s, 9 H, 3 CH_3) ppm. ^{13}C NMR (125.75 MHz, CD_3OD): δ = 184.42 (6 C=S), 83.70 (3 C-1), 77.93 (3 C-5), 75.07, 74.54 (3 C-3), 72.15, 72.03, 71.39 (3 C-2), 69.56, 68.98, 68.78 (3 C-4), 54.44, 54.09 (3 NCH_2), 46.30 (3 C-6), 44.11 (3 NCH_2CH_2), 26.81 [3 C(NN)], 18.34, 18.28 (3 CH_3) ppm. ESI MS: m/z = 1188.3929 [M + H]$^+$ (calcd. m/z = 1188.3770) for $C_{39}H_{70}N_{19}O_{12}S_6$; m/z = 1210.3760 [M + Na]$^+$ (calcd. m/z = 1210.3590) for $C_{39}H_{69}N_{19}NaO_{12}S_6$.

5-Azidomethyl-2,2-dimethyl-1,3-dioxane (7): 2-Hydroxymethyl-1,3-propandiol (254 mg, 2.4 mmol) was dissolved in dry THF (5 mL) and mixed with 2,2-dimethoxypropane (440 μL, 374 mg, 3.6 mmol) and acetone (264 μL, 209 mg, 3.6 mmol). The reaction was started by the addition of *p*-toluenesulfonic acid (50 mg) and the mixture was stirred for 1.5 h at room temp. The reaction was quenched by the addition of solid sodium carbonate (one spoon), finally the liquid phase was filtered and concentrated. The residue was taken up in dichloromethane/water, the aqueous phase was extracted twice with dichloromethane, and the combined organic phases were washed with water, dried with sodium sulfate and filtered. Without further purification the concentrated residue was dissolved in dry pyridine (10 mL), tosyl chloride (503 mg, 2.6 mmol) was added and the reaction mixture was stirred overnight at room temp. Then methanol (1 mL) was added and the solution concentrated in vacuo. The residue was taken up in dichloromethane/water. The aqueous phase was extracted with dichloromethane once, the combined organic phases were washed with water, dried with sodium sulfate and filtered. The concentrated residue was then dissolved in dry toluene (10 mL), sodium azide (780 mg, 0.012 mol) and TBABr (773 mg, 2.4 mmol) were added and the reaction mixture was stirred overnight at 65 °C. The mixture was concentrated, the residue taken up in dichloromethane/water, the aqueous phase extracted with dichloromethane and the combined organic phases were washed with water, dried with sodium sulfate and filtered. Column chromatography (cyclohexane/ethyl acetate, 3:1) gave **7** (170 mg, 0.99 mmol, 41%) as a colorless syrup. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.02 (dd, 2 H, H_{eq}, $H_{eq'}$), 3.71 (dd, 2 H, H_{ax}, $H_{ax'}$), 3.50 (d, 2 H, CH_2N_3), 1.82 (dsept, 1 H, CH), 1.44 (s, 3 H, CH_3), 1.40 (s, 3 H, CH_3) ppm; $^3J_{CHCHaxHeq}$ = 5.2, $^3J_{CHCHaxHeq}$ = 3.7, $^3J_{CHCHHN}$ = 7.3, $^2J_{CHaxHeq}$ = $^2J_{CHax'Heq'}$ = 12.1 Hz. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 98.23 (C_q), 61.55 (2 CH_2O), 50.81 (CH_2N_3), 34.18 (CH), 25.15, 22.42 (2 CH_3) ppm. MS (CI): m/z = 172.1 [M + H]$^+$ (calcd. m/z = 172.2) for $C_7H_{14}N_3O_3$, m/z = 144.1 [M + H – N_2]$^+$ (calcd. m/z = 144.2) for $C_7H_{15}NO_2$.

2-Azidomethyl-1,3-propanediol (8): The dioxane **7** (204 mg, 1.2 mmol) was dissolved in MeOH (1 mL), mixed with concd. hydrochloric acid (200 μL) and stirred at room temp. for 1 h. After complete deprotection the solution was neutralized with ion-exchange resin (Merck III-OH$^-$), filtered and the solution concentrated to provide **8** as a colorless syrup (156 mg, 1.2 mmol, quant.). ^{1}H NMR (300 MHz, CD_3OD): δ = 3.63 (t, 4 H, 2 C*H*$_2$OH), 3.48 (d, 2 H, CH_2N_3), 1.90 (sept, 1 H, CH) ppm. ^{13}C NMR (75.47 MHz, CD_3OD): δ = 61.26 (2 CH_2OH), 50.91 (CH_2N_3), 45.03 (CH) ppm. MS (CI): m/z = 132.1 [M + H]$^+$ (calcd. m/z = 132.1) for $C_4H_{10}N_3O_2$, 104.0 [M + H – N_2]$^+$ (calcd. m/z = 104.1) for $C_4H_{10}NO_2$.

1,3-Bis(2′,3′,4′,6′-tetra-*O*-benzoyl-α-D-mannopyranosyloxy)-2-(azidomethyl)propane (10): The azide **8** (87 mg, 0.66 mmol) and the mannosyl donor **9** (1.09 g, 1.46 mmol) were dissolved in dry dichloromethane (10 mL) under argon atmosphere at 0 °C. The glycosylation reaction was started by the addition of TMS-OTf (10μL). The solution was stirred at 0 °C for 1 h and then for another hour at room temp. After quenching the reaction by addition of concd. sodium hydrogen carbonate solution (1 mL) the mixture was diluted with dichloromethane/water (1:1, 50 mL). The organic phase was separated and dried with sodium sulfate. After filtration and concentration, column chromatography (cyclohexane/ethyl acetate, 3:1) gave **10** as a colorless solid (637 mg, 0.49 mmol, 75%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.11, 8.03, 7.96, 7.81 (each dd, each 4 H, 16 H^{Bz}), 7.61–7.21 (m, 24 H, H^{Bz}), 6.14 (t, $^3J_{4,5}$ = 10.1 Hz, 2 H, H-4^{man1}, H-4^{man2}), 5.93–5.88 (m, $^3J_{3,4}$ = 10.1 Hz, 2 H, H-3^{man1}, H-3^{man2}), 5.77–5.74 (m, $^3J_{2,3}$ = 3.3 Hz, 2 H, H-2^{man1}, H-2^{man2}), 5.17 (s, $^3J_{1,2}$ = 1.8 Hz, 2 H, H-1^{man1}, H-1^{man2}), 4.77, 4.75 (each dd, $^2J_{6,6'}$ = 12.2 Hz, each 1 H, H-6′^{man1}, H-6′^{man2}), 4.57, 4.55 (each dd, each 1 H, H-6^{man1}, H-6^{man2}), 4.50–4.44 (m, $^3J_{5,6}$ = 4.4, $^3J_{5,6'}$ = 3.9 Hz, 2 H, H-5^{man1}, H-5^{man2}), 4.02 (dd, 1 H, C*H*HO^{man1}*), 3.97 (C*H*HO^{man2}*), 3.74–3.64 (m, 4 H, CH_2N_3, CH*H*O^{man1}, CH*H*O^{man2}), 2.44 [sept, 1 H, C*H*(CH_2)$_3$] ppm. ^{13}C NMR (125.75 MHz, $CDCl_3$): δ = 166.15, 165.53, 165.38, 165.35 (8 C=O), 133.48, 133.42, 133.19, 133.11 [8 C(O)C^{Bz}], 129.89, 129.84, 129.74, 129.73, 129.40, 129.24, 129.02, 128.58, 128.48, 128.42, 128.30 (C^{Bz}), 98.07 (C-1^{man}, C-1^{man2}), 70.29 (C-2^{man1}, C-2^{man2}), 70.13, 70.10 (C-3^{man1}, C-3^{man2}), 69.30 (C-5^{man1}, C-5^{man2}), 66.74 (C-4^{man1}, C-4^{man2}), 66.38, 66.30 (CH_2O^{man1}, CH_2^{man}2), 62.83 (C-6^{man1}, C-6^{man2}), 49.88 (CH_2N_3), 39.17 [*C*H(CH_2)$_3$] ppm. MALDI-TOF MS (norharmane): m/z = 1283.1 [M + Na – N_2]$^+$ (calcd. m/z = 1283.24) for $C_{72}H_{61}NNaO_{20}$; m/z = 1299.0 [M + K – N_2]$^+$ (calcd. m/z = 1299.19) for $C_{72}H_{61}KNO_{20}$.

1,3-Bis(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyloxy)-2-(isothiocyanatomethyl)propane (11): The azide **10** (300 mg, 0.23 mmol) was dissolved in dry toluene (8 mL) and mixed with carbon disulfide (560 μL, 707 mg, 9.3 mmol) and triethyl phosphite (160 μL, 154 mg, 0.93 mmol). The solution was heated to 80 °C and stirred under reflux for 8 h. After cooling to 0 °C water (5 mL) was added

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and the reaction mixture was left standing overnight at 4 °C. Dichloromethane (20 mL) was added, the organic phase was separated, the aqueous phase was extracted with dichloromethane and the combined organic phases were dried with sodium sulfate. After filtration and concentration, flash column chromatography (cyclohexane/ethyl acetate, 3:1) yielded **11** (170 mg, 0.13 mmol, 56%) as a yellowish solid. $[\alpha]_D^{20}$ = –16.1 (c = 0.88, $CHCl_3$). IR (KBr): $\tilde{\nu}_{NCS}$ = 2104.4 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 8.07–7.14 (m, 40 H, H^{Bz}), 6.15–6.06 (m, $^3J_{4,5}$ = 10.1 Hz, 2 H, H-4^{man1}, H-4^{man2}), 5.81 (dd, $^3J_{3,4}$ = 10.1 Hz, 2 H, H-3^{man1}, H-3^{man2}), 5.68 (dd, $^3J_{2,3}$ = 3.2 Hz, 2 H, H-2^{man1}, H-2^{man2}), 5.11 (d, $^3J_{1,2}$ = 1.5 Hz, 1 H, H-1^{man1}*), 5.09 (d, 1 H, H-1^{man2}*), 4.74 –4.63 (m, $^3J_{6',6}$ = 12.3 Hz, 2 H, H-6′^{man1}, H-6′^{man2}), 4.52–4.45 (m, 2 H, H-6^{man1}, H-6^{man2}), 4.42–4.37 (m, $^3J_{5,6'}$ = 2.5 Hz, 2 H, H-5^{man1}, H-5^{man2}), 4.02–3.88 (m, 3 H, CH_2O^{man1}*, C*H*H–NCS), 3.83–3.77 (m, 1 H, CH*H*–NCS), 3.63 (dd, 1 H, CH_2O^{man2}*), 2.50 (br. quint, 1 H, CH) ppm. ^{13}C NMR (125.75 MHz, $CDCl_3$): δ = 165.54, 165.52, 165.51, 165.48, 165.34, 165.33 (8 C=O), 133.49, 133.44, 133.40, 133.21, 133.12, 133.09, 130.02, 129.94, 129.87, 129.85, 129.75, 129.71, 129.18, 129.17, 128.98, 128.95, 128.89, 128.62, 128.57, 128.49,128.42, 128.41, 128.31, 128.29 (C_{Bz}), 98.18, 98.09 (C-1^{man1}, C-1^{man2}), 70.18, 70.15 (C-3^{man1}, C-3^{man2}), 70.11, 70.05 (C-2^{man1}, C-2^{man2}), 69.41, 69.39 (C-5^{man1}, C-5^{man2}), 66.59, 66.52 (C-4^{man1}, C-4^{man2}), 65.90, 65.85 (CH_2O^{man1}, CH_2O^{man2}), 62.70, 62.66 (C-6^{man1}, C-6^{man2}), 43.76 (CH_2NCS), 39.72 (CH) ppm. MALDI-TOF MS: m/z = 1327.0 $[M + Na]^+$ (calcd. m/z = 1326.34) for $C_{73}H_{61}NNaO_{20}S$; m/z = 1342.9 $[M + K]^+$ (calcd. m/z = 1342.29 for $C_{73}H_{61}KNO_{20}S$).

2-(2′-Azipropylthiourenyl)-1,3-bis(2′′,3′′,4′′,6′′-tetra-*O*-benzoyl-α-D-mannopyranosyloxy)propane (12): The isothiocyanate **11** (149 mg, 0.11 mmol) was dissolved in dry dichloromethane (2 mL) and mixed with **4** (14 mg, 0.11 mmol) and a solution (50 μL) of DIPEA in dry dichloromethane (0.5 mL). The reaction mixture was strirred at room temp. for 4 h, then the solvent was removed in vacuo. Purification of the residual syrup by column chromatography (cyclohexane/ethyl acetate, 2:1) yielded **12** (76 mg, 0.06 mmol, 48%) as a colorless solid. $[\alpha]_D^{20}$ = –14.6 (c = 0.52, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 8.13–7.15 (m, 40 H, H^{Bz}), 6.81 ($NH^{diazirine}$), 6.62 (NH–CH_2^{core}), 6.22 (t, $^3J_{4,5}$ = 10.2 Hz, 2 H, H-4^{man1}, H-4^{man2}), 5.88 (dd, $^3J_{3,4}$ = 10.2 Hz, 1 H, H-3^{man1}*), 5.82–5.75 (m, $^3J_{2,3}$ = 3.1 Hz, 3 H, H-2^{man1}, H-2^{man2}, H-3^{man2}*), 5.20 (d, $^3J_{1,2}$ = 1.5 Hz, 1 H, H-1^{man1}*), 5.18 (d, 1 H, H-1^{man2}*), 4.83–4.73 (m, $^2J_{6,6'}$ = 12.1 Hz, 2 H, H-6′^{man1}, H-6′^{man2}), 4.63–4.54 (m, $^3J_{5,6}$ = 3.9, $^3J_{5,6'}$ = 2.4 Hz, 4 H, H-5^{man1}, H-5^{man2}, H-6^{man1}, H-6^{man2}), 4.36–4.26 [m, 1 H, NHC*H*HC(NN)], 4.21 (dd, 1 H, C*H*HO^{man1}*), 4.12 (dd, 1 H, C*H*HO^{man2}*), 3.97–3.89 [m, 1 H, NHCH*H*C(NN)], 3.83–3.74 (m, 3 H, CH*H*O^{man1}, CH*H*O^{man2}, C*H*HNH), 3.59 (dd, 1 H, CH*H*NH), 2.56 (sept, 1 H, CH), 1.06 (s, 3 H, CH_3) ppm. ^{13}C NMR (125.75 MHz, $CDCl_3$): δ = 184.10 (C=S), 166.27, 166.20, 166.16, 165.54, 165.51, 165.46 (8 C=O), 133.55, 133.49, 133.38, 133.36, 133.26, 133.13, 133.07, 129.90, 129.88, 129.77, 129.76, 129.71, 129.14, 129.04, 128.95, 128.87, 128.58, 128.57, 128.51, 128.48, 128.46, 128.37, 128.30, 128.29 (C^{Bz}), 98.09, 98.01 (C-1^{man1}, C-1^{man2}), 70.74, 70.68 (C-3^{man1}, C-3^{man2}), 70.34, 70.22 (C-2^{man1}, C-2^{man2}), 69.87 (CH_2O^{man1}*), 69.61, 69.46 (C-5^{man1}, C-5^{man2}), 69.28 (CH_2O^{man2}*), 66.30, 66.26 (C-4^{man1}, C-4^{man2}), 62.76, 62.73 (C-6^{man1}, C-6^{man2}), 47.72 (CH_2NH^{core}), 46.43 [NH*C*H$_2$C(NN)], 38.81 (CH), 25.85 [C(NN)], 18.19 (CH_3) ppm. MALDI-TOF MS (norharmane): m/z = 1384.7 $[M + Na – N_2]^+$ (calcd. m/z = 1384.41) for $C_{76}H_{68}N_2NaO_{20}S$, m/z = 1361.7 $[M – N_2]^+$ (calcd. m/z = 1361.47) for $C_{76}H_{68}N_2O_{20}S$.

[2-(2′-Azipropylthiourenyl)-1,3-bis(α-D-mannopyranosyloxy)]-propane (13): The protected derivative **12** (65 mg, 47μmol) was dissolved in a 1:1 mixture of MeOH and THF (10 mL) and mixed with sodium methoxide solution (1 M in MeOH, 37 μL). The reaction mixture was stirred for 1 h at room temp., neutralized with Amberlite ion-exchange resin IR 120, filtered and purified by HPLC (Merck RP-8, 7 μm; MeCN/H_2O, 10:90). After lyophilisation **13** was obtained as a colorless foam (17 mg, 30 μmol, 65%). ^1H NMR (500 MHz, D_2O): δ = 4.83 (br. s, 2 H, 2 H-1), 4.02–3.43 (m, 20 H, 2 H-2, 2 H-3, 2 H-4, 2 H-5, 2 H-6, 2 H-6′, OCH_2^{man}, CH_2NH), 2.32 (br. s, 1 H, CH), 1.06 (s, 3 H, CH_3) ppm. ^{13}C NMR (125.75, D_2O): δ = 102.42 (C-1), 75.25 (C-5), 73.03 (C-3), 72.41 (C-2), 69.18 (C-4), 68.77, 68.62 (OCH_2^{man}), 63.30 (C-6), 40.90 (CH), 19.32 (CH_3) ppm. MALDI-TOF MS (DHB): m/z = 551.4 $[M + Na – N_2]^+$ (calcd. m/z = 551.57) for $C_{20}H_{36}N_2NaO_{12}S$; m/z = 529.3 $[M – N_2]^+$ (calcd. m/z = 528.58) for $C_{20}H_{36}N_2O_{12}S$. ESI MS: m/z = 579.1985 $[M + Na]^+$ (calcd. m/z = 579.1943) for $C_{20}H_{36}N_4NaO_{12}S$.

1,3-Bis(2′,3′,4′,6′-tetra-*O*-benzoyl-α-D-mannopyranosyloxy)-2-(2′′-azipropylthiourenyl-2′′′,3′′′,4′′′-tri-*O*-acetyl-6′′′-deoxy-6′′′-thiourenylmethyl-α-D-mannopyranos-6′′′-yl)propane (16): The azide **10** (600 mg, 0.47 mmol) was dissolved in dry THF (5 mL) under argon atmosphere at 0 °C and mixed with a solution of triphenylphosphane (244 mg, 0.93 mmol) in dry THF (2 mL). The mixture was stirred at 0 °C for 0.5 h, then at room temp. for another 0.5 h. The diazirine-labeled mannose derivative **15** (232 mg, 0.49 mmol), dissolved in dry THF (2 mL) and DIPEA (50 μL), was added to the reaction mixture together with water (2 mL). The solution was stirred at room temp. for 5 h, then concentrated in vacuo. Two purification steps (flash column chromatography first with cyclohexane/ethyl acetate, 2:1 → 3:2, then cyclohexane/ethyl acetate, 1:1) led to the pure product (188 mg, 0.11 mmol, 23%) as an amorphous solid. $[\alpha]_D^{20}$ = –4.1 (c = 1.25, H_2O). ^1H NMR (500 MHz, $CDCl_3$): δ = 8.18–7.27 (m, 40 H, H^{Bz}), 8.11 (br. s, 1 H, NH), 7.20 (br. s, 1 H, NH), 6.29–6.22 (m, 2 H, H-4^{man1}, H-4$_{Bz}^{man2}$), 6.11 (br. s, 1 H, H-1^{man3}), 6.03–5.97 (m, 2 H, H-3^{man1}, H-3^{man2}), 5.91–5.87 (m, 2 H, H-2^{man1}, H-2^{man2}), 5.38–5.30 (m, 3 H, H-1^{man1}, H-1^{man2}, H-3^{man3}), 5.27 (t≈dd, 1 H, H-2^{man3}), 5.07 (t, 1 H, H-4^{man3}), 4.87–5.75 (m, 4 H, H-6′^{man1}, H-6′^{man2}, H-5^{man1}, H-5^{man2}), 4.70–4.62 (m, 2 H, H-6^{man1}, H-6^{man2}), 4.27–4.19 (m, 2 H, C*H*HO^{man1}, C*H*HO^{man2}), 4.06–3.90 (m, 6 H, H-5^{man3}, H-6^{man3}, H-6′^{man3}, CH–C*H*HNH, CH*H*O^{man1}, CH*H*O^{man2}), 3.77–3.63 [m, 3 H, $CH_2C(NN)$, CH–CH*H*NH], 2.73 (sept, 1 H, CH), 2.04, 1.98, 1.95 [each br. s, each 3 H, 3 C(O)CH_3], 1.04 (s, 3 H, CH_3) ppm; man1,2: $^3J_{1,2}$ = 1.6, $^3J_{2,3}$ = 3.3, $^3J_{3,4}$ = 9.9, $^3J_{4,5}$ = 9.9; man3: $^3J_{2,3}$ = 3.4, $^3J_{3,4}$ = 7.9, $^3J_{4,5}$ = 7.9; core: $^3J_{CHCHH}$ = 5.7 Hz. ^{13}C NMR (125.75 MHz, $CDCl_3$): δ = 170.22, 170.13, 170.09 (3 C=O^{Ac}), 166.43, 166.39, 166.01, 165.86, 165.85, 165.72, 165.71 (8 C=O^{Bz}), 134.40, 134.19, 134.15, 134.09, 134.04, 134.02, 131.05, 130.43, 130.42, 130.40, 130.37, 130.36, 130.34, 130.19, 130.18, 130.15, 129.57, 129.51, 129.30, 129.29, 129.21, 129.19 (C^{Bz}), 99.09, 98.97 (C-1^{man1}, C-1^{man1}), 79.97 (C-1^{man3}), 71.66 (C-3^{man1}, C-3^{man2}), 71.23, 71.15 (C-2^{man1}, C-2^{man2}), 69.97, 69.92 (C-5^{man1}, C-5^{man2}), 69.74 (C-2^{man3}), 69.40 (C-3^{man3}), 68.54 (C-4^{man3}), 67.95, 67.88 (CH_2O^{man1}, CH_2O^{man2}), 67.29 (C-4^{man1}, C-4^{man1}), 63.35 (C-6^{man1}, C-6^{man2}), 47.76 [$CH_2C(NN)$], 45.29 (C-6^{man3}), 43.44 (CH*C*H$_2$NH), 40.10 (CH), 26.45 [C(NN)], 20.72, 20.64, 20.53 [3 C(O)CH_3], 18.30 (CH_3) ppm.

1,3-Bis(α-D-mannopyranosyloxy)-2-(2′-azipropylthiourenyl-6′′-deoxy-6′′-thiourenylmethyl-α-D-mannopyranos-6′′-yl)propane (17): The protected cluster **16** (180 mg, 0.1 mmol) was dissolved in dry MeOH (2 mL) and sodium methoxide (1 M in MeOH, 114 μL) was added. The reaction mixture was stirred at room temp. or 1 h, then the solution was neutralized with Amberlite ion exchanger IR 120, filtered, and concentrated. The residue was purified by HPLC (Merck RP-8, 7 μm; MeCN/H_2O, 15:85). Lyophilisation led to **17**

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(51 mg, 0.07 mmol, 63%) as a colorless foam. $[\alpha]_D^{20}$ = +39.8 (*c* = 0.88, H_2O). ^{1}H NMR (500 MHz, D_2O): δ = 5.50 (br. s, 1 H, NH), 4.86–4.81 (m, 2 H, H-1^{man3}, H-1^{man3}*), 4.02–3.93 (m, 3 H, H-2^{man1}, H-2^{man2}, H-2^{man3}), 3.87 (d≈dd, 2 H, H-6′^{man1}, H-6′^{man2}), 3.84–3.47 [m, 21 H, H-3^{man1}, H-3^{man2}, H-3^{man3}, H-4^{man1}, H-4^{man2}, H-4^{man3}, H-5^{man1}, H-5^{man2}, H-5^{man3}, H-6^{man1}, H-6^{man2}, H-6^{man3}, H-6′^{man3}, CH_2O^{man1}, CH_2O^{man2}, CHCH_2NH, CH_2C(NN)], 2.33 (br. s, 1 H, CH), 1.07 (br. s, 3 H, CH_3) ppm; man1,2: $^3J_{1,2}$ = 1.6, $^3J_{2,3}$ = 3.3, $^3J_{3,4}$ = 9.7, $^3J_{4,5}$ = 9.7, $^3J_{5,6}$ = 5.7, $^2J_{6,6'}$ = 11.9 Hz. ^{13}C NMR (125.75 MHz, D_2O): δ = 102.46, 102.44 (C-1^{man1}, C-1^{man2}), 84.44 (C-1^{man3}), 75.33 (C-5^{man3}), 75.25 (C-5^{man1}, C-5^{man2}), 73.04 (C-3^{man1}, C-3^{man2}), 72.67 (C-3^{man3}), 73.43 (C-2^{man1}, C-2^{man2}), 70.05 (C-4^{man3}), 69.17 (C-4^{man1}, C-4^{man2}), 68.78, 68.68 (CH_2O^{man1}, CH_2O^{man2}), 63.29 (C-6^{man1}, C-6^{man2}), 48.57 [*C*H$_2$C(NN)], 46.40 (C-6^{man3}), 40.98 (CH), 28.86 [C(NN)], 19.46 (CH_3) ppm. ESI MS: *m*/*z* = 799.2506 $[M + Na]^+$ (calcd. *m*/*z* = 799.2460) for $C_{27}H_{48}N_6NaO_{16}S_2$.

Tri-*O*-allyl-*O*-tosylpentaerythritol (19): Triallyl pentaerythritol (**18**, 4.00 g, 16 mmol) was dissolved in dry pyridine (30 mL). Tosyl chloride (3.24 g, 17 mmol) was added in portions to this solution and the mixture stirred at room temp. overnight. The reaction was quenched by addition of methanol (10 mL), the solution concentrated and the residue taken up in dichloromethane/concd. aqueous sodium hydrogen carbonate solution. The organic phase was separated, washed once with concd. aqueous sodium hydrogen carbonate solution and dried with sodium sulfate. After filtration and column chromatography (cyclohexane/ethyl acetate, 9:1) **19** was obtained (5.30 g, 13 mmol, 81%) as a yellowish syrup. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.77 (d, 2 H, $H_{a,a'}$), 7.33 (d, 2 H, $H_{x,x'}$), 5.79 (dddd, 3 H, 3 OCH_2CH=CH_2), 5.18 (ddd≈dd, $^2J_{gem}$ = 1.8 Hz, $^3J_{trans}$ = 17.3 Hz, 3 H, 3 OCH_2CH=CHH_t), 5.12 (ddd≈dd, $^3J_{cis}$ = 10.5 Hz, 3 H, 3 OCH_2CH=CH$_c$H), 4.06 (s, 2 H, $TsOCH_2$), 3.86 (dt, $^3J_{OCHHCH}$ = 5.4 Hz, 6 H, 3 OCH_2CH=CH_2), 3.38 [s, 6 H, 3 $C(CH_2O)_3$], 2.44 (CH_3) ppm. ^{13}C NMR (125.75 MHz, $CDCl_3$): δ = 144.53 (H_3CC_{aryl}), 134.76 (3 *C*H=CH_2), 132.90 (O_3SC_{aryl}), 129.73 [$H_3CC_{aryl}(C_{x,x'})$], 127.96 [$O_3SC_{aryl}(C_{a,a'})$], 116.39 (3 CH=*C*H$_2$), 72.28 (3 O*C*H$_2$CH=CH_2), 69.74 ($TsOCH_2$), 68.18 [3 C(*C*H$_2$O)$_3$], 44.81 (C_q), 21.61 (CH_3) ppm.

Tri-*O*-(2-hydroxyethyl)-*O*-tosylpentaerythritol (20): The tosylate **19** (5.00 g, 12 mmol) was dissolved in a 1:1 mixture of dry dichloromethane and dry methanol (40 mL), the solution was cooled to –78 °C. Oxygen was passed through the solution for 5 min, then ozone for 40 min, then again oxygen for 5 min and finally argon for 5 min. Then sodium boronhydride (4.54 g, 0.12 mol) was added in portions to the cold solution. The reaction mixture was stirred for 2 h at 0 °C and was then left standing at room temp. overnight. The reaction was quenched by addition of acetic acid (15 mL), the solution concentrated and the residue taken up in diethyl ether/water. The aqueous phase was extracted twice with diethyl ether and the combined organic phases were washed once with water. After drying with sodium sulfate and filtration, column chromatography (ethyl acetate/cyclohexane, 2:1 → ethyl acetate → ethyl acetate/methanol, 2:1) the triol **20** (2.32 g, 5.0 mmol, 46%) was obtained as a colorless syrup. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.78 (d, $^3J_{a,x}$ = $^3J_{a',x'}$ = 8.3 Hz, 2 H, $H_{a,a'}$), 7.36 (d, 2 H, $H_{x,x'}$), 4.07 (s, 2 H, CH_2OTs), 3.69–3.63 (m, 6 H, 3 CH_2OH), 3.53–3.47 (m, 6 H, 3 CH_2CH$_2$OH), 3.45 [s, 6 H, 3 $C(CH_2O)_3$], 3.02 (br. s, 3 H, 3 OH), 2.46 (s, 3 H, CH_3) ppm. ^{13}C NMR (125.75 MHz, $CDCl_3$): δ = 145.01 (H_3CC_{aryl}), 132.71 (O_3SC_{aryl}), 129.91 [$H_3CC_{aryl}(C_{x,x'})$], 127.91 [$O_3SC_{aryl}(C_{a,a'})$], 72.67 (3 O*C*H$_2$CH$_2$OH), 69.10 ($TsOCH_2$), 69.04 [C(*C*H$_2$O)$_3$], 61.37 (3 OCH$_2$*C*H$_2$OH), 44.95 (C_q), 21.65 (CH_3) ppm.

Tri-*O*-(2-acetoxyethyl)-*O*-tosylpentaerythritol (21): To a solution of the triol **20** (2.20 g, 5.0 mmol) in dry pyridine (20 mL) acetic anhydride (2.8 mL, 0.03 mol) was added and the reaction mixture was stirred at room temperature for 4 h. Then it was concentrated, washed with concd. aqueous sodium hydrogen carbonate solution, dried with sodium sulfate and filtered. Purification by column chromatography (cyclohexane/ethyl acetate, 3:2) yielded **21** (2.40 g, 4.0 mmol, 84%) as a colorless syrup. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.78 (d, $^3J_{a,x}$ = $^3J_{a',x'}$ = 8.3 Hz, 2 H, $H_{a,a'}$), 7.35 (d, 2 H, $H_{x,x'}$), 4.14–4.08 (m, 6 H,3 CH_2OAc), 4.03 (s, 2 H, CH_2OTs), 3.56–3.50 (m, 6 H, 3 CH_2CH$_2$OAc), 3.40 [s, 6 H, $C(CH_2O)_3$], 2.46 (s, 3 H, CH_3), 2.06 [s, 9 H, 3 C(O)*C*H$_3$] ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 170.96 (3 C=O), 144.79 (H_3CC_{aryl}), 132.86 (O_3SC_{aryl}), 129.79 [$H_3CC_{aryl}(C_{x,x'})$], 127.94 [$O_3SC_{aryl}(C_{a,a'})$], 69.22 (3 O*C*H$_2$-CH$_2$OAc), 69.16 ($TsOCH_2$), 68.72 [C(*C*H$_2$O)$_3$], 61.37 (3 OCH$_2$-*C*H$_2$OAc), 44.95 (C_q), 21.65 (H_3CC_{aryl}), 20.90 [3 C(O)*C*H$_3$] ppm. MALDI-TOF MS: *m*/*z* = 571.5 $[M + Na]^+$ (calcd. *m*/*z* = 571.18), 587.5 $[M + K]^+$ (calcd. *m*/*z* = 587.15) for $C_{24}H_{36}O_{12}S$.

1-Azido-3-(2′-acetoxyethoxy)-2,2-bis(2′-acetoxyethoxymethyl)propane (22): To a solution of the tosylate **21** (2.20 g, 4.0 mmol) in dry DMF (15 mL) sodium azide (1.30 g, 0.02 mol) and TBABr (1.29 g, 4.0 mmol) were added. The reaction mixture was stirred for 6 d at 85 °C. The solution was concentrated and the residue taken up in water/ethyl acetate. The organic phase was separated, washed twice with water, dried with sodium sulfate and filtered. After concentration in vacuo and column chromatography (cyclohexane/ethyl acetate, 2:1) **22** was obtained (1.57 g, 3.7 mmol, 93%) as a colorless syrup. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.20 (dt, 6 H, 3 CH_2OAc), 3.61 (dt, 6 H, 3 OCH_2CH_2OAc), 3.40 [s, 6 H, 3 $C(CH_2O)_3$], 3.35 (s, 2 H, CH_2N_3), 2.07 [s, 9 H, 3 C(O)CH_3] ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 171.00 (3 C=O), 69.53 [C(*C*H$_2$O)$_3$], 69.27 (3 O*C*H$_2$CH$_2$OAc), 63.34 (3 CH_2OAc), 51.50 (CH_2N_3), 45.49 (C_q), 20.93 [3 C(O)*C*H$_3$] ppm. MS (CI): *m*/*z* = 392.2 $[M + H - N_2]^+$ (calcd. *m*/*z* = 392.19) for $C_{17}H_{30}NO_9$.

1-Azido-3-(2′-hydroxyethoxy)-2,2-bis(2′-hydroxyethoxymethyl)propane (23): The azide **22** (1.50 g, 3.6 mmol) was dissolved in dry MeOH (5 mL), mixed with sodium methoxide (1 M in MeOH, 1 mL) and stirred at room temp. for 30 min. Then the solution was neutralized with Amberlite ion-exchange resin IR 120. After filtration, removal of the solvent and purification by column chromatography (ethyl acetate/cyclohexane, 2:1 → MeOH/ethyl acetate, 2:1) **23** was obtained (952 mg, 3.3 mmol, 91%) as a yellowish syrup. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.76–3.69 (m, 6 H, 3 CH_2OH), 3.61–3.54 (m, 6 H, 3 OCH_2CH$_2$OH), 3.46 [s, 6 H, $C(CH_2O)_3$], 3.39 (s, 2 H, CH_2N_3), 2.98 (br. s, 3 H, 3 OH) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 72.63 (3 O*C*H$_2$CH$_2$OH), 70.02 [C(*C*H$_2$O)$_3$], 61.41 (3 CH_2OH), 51.91 (CH_2N_3), 45.34 (C_q) ppm.

1-Azido-3-[2′-(2″,3″,4″,6″-tetra-*O*-benzoyl-α-D-mannopyranosyloxy)ethoxy]-2,2-bis[2′-(2″,3″,4″,6″-tetra-*O*-benzoyl-α-D-mannopyranosyloxy)ethoxymethyl]propane (24): The triol **23** (100 mg, 0.34 mmol) and **9** (833 mg, 1.12 mmol) were dissolved in dry dichloromethane (10 mL) under argon and cooled to 0 °C. The reaction was started by addition of TMS-OTf (10μL). The mixture was stirred at 0 °C for 1 h, then for an additional day at room temp. The reaction was quenched by addition of a small amount of concd. aqueous sodium hydrogen carbonate solution. The mixture was taken up in dichloromethane/water, the organic phase separated, dried with sodium sulfate and filtered. After removal of the solvent and purification by column chromatography (cyclohexane/ethyl acetate, 5:2) **24** was obtained (466 mg, 0.23 mmol, 67%) as a colorless solid. $[\alpha]_D^{20}$ = –20.7 (*c* = 1.98, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.10, 8.03, 7.93, 7.82 (each dd, each 4 H,

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16 H^{Bz}), 7.58–7.53 (m, 4 H, 4 H^{Bz}), 7.47–7.29 (m, 24 H, H^{Bz}), 7.25–7.21 (m, 6 H, 6 H^{Bz}), 6.13 (t, 3 H, 3 H-4), 5.96 (dd, 3 H, 3 H-3), 5.73 (dd, 3 H, 3 H-2), 5.17 (d, 3 H, 3 H-1), 4.72 (dd, 3 H, 3 H-6′), 4.54–4.47 (m, 6 H, 3 H-5, 3 H-6), 3.94 (ddd, 3 H, 3 manOC*H*HCH$_2$), 3.79 (ddd, 3 H, 3 manOCH*H*CH$_2$), 3.73 (q, 6 H, 3 manOCH$_2$C*H*$_2$), 3.57 [d, 6 H, 3 C(CH$_2$O)$_3$], 3.47 (d, 2 H, CH$_2$N$_3$) ppm; $^3J_{a,x} = {}^3J_{a',x'} = 8.4$, $^3J_{1,2} = 1.8$, $^3J_{2,3} = 3.3$, $^3J_{3,4} = 10.1$, $^3J_{4,5} = 10.1$, $^3J_{5,6} = 4.2$, $^3J_{5,6'} = 2.2$, $^2J_{6,6'} = 11.5$, $^2J_{CHHCHH\text{Oman}} = 11.2$, $^3J_{CHHCHH\text{Oman}} = 5.6$, $^3J_{CHHCHH\text{Oman}} = 8.8$ Hz. ^{13}C NMR (125.75 MHz, CDCl$_3$): δ = 165.14, 165.49, 165.38, 165.30 (12 C=O), 133.40, 133.37, 133.07, 133.03 [OC(O)C^{Bz}], 129.94, 129.84, 129.75, 129.73, 129.40, 129.17, 129.04, 128.55, 128.45, 128.43, 128.27 (C^{Bz}), 97.66 (3 C-1), 70.50 (3 manOCH$_2$*C*H$_2$), 70.48 (3 C-2), 70.11 (3 C-3), 69.91 [C(*C*H$_2$O)$_3$], 68.79 (3 C-5), 67.17 (3 manO*C*H$_2$CH$_2$), 67.00 (3 C-4), 62.87 (3 C-6), 51.90 (CH$_2$N$_3$), 45.73 (C$_q$) ppm. MALDI-TOF MS (norharmane): *m*/*z* = 2052.3 [M – Na]$^+$ (calcd. *m*/*z* = 2052.0) for $C_{117}H_{108}N_2NaO_{33}S$; *m*/*z* = 2068.2 [M + K]$^+$ (calcd. 2068.0) for $C_{117}H_{108}KN_2O_{33}S$.

1-Isothiocyanato-3-[2′-(2′′,3′′,4′′,6′′-tetra-*O*-benzoyl-α-D-mannopyranosyloxy)ethoxy]-2,2-bis[2′-(2′′,3′′,4′′,6′′-tetra-*O*-benzoyl-α-D-mannopyranosyloxy)ethoxymethyl]propan (25): The azide **24** (400 mg, 0.20 mmol) was dissolved in dry toluene (5 mL) and mixed with carbon disulfide (480 μL, 600 mg, 8 mmol) and triethyl phosphite (136 μL, 131 mg, 0.80 mmol). The reaction mixture was heated to 80 °C and stirred at reflux temperature for 8 h. After cooling, water (2 mL) was added and the reaction mixture was left standing overnight at 4 °C. The organic phase was diluted with dichloromethane and the aqueous phase extracted once with dichloromethane. The combined organic phases were dried with sodium sulfate. The solution was filtered, concentrated and the residue purified by column chromatography (cyclohexane/ethyl acetate, 3:2) to yield **25** (333 mg, 0.16 mmol, 83%) as a colorless solid. $[\alpha]_D^{20} = -41.4$ (*c* = 0.64, CHCl$_3$). ^{1}H NMR (500 MHz, CDCl$_3$): δ = 8.09, 8.03, 7.93, 7.82 (each dd, each 4 H, H^{Bz}), 7.58–7.52 (m, 4 H, 4 H^{Bz}), 7.47–7.29 (m, 24 H, H^{Bz}), 7.25–7.20 (t, 6 H, 6 H^{Bz}), 6.13 (t, $^3J_{4,5}$ = 10.0 Hz, 3 H, 3 H-4), 5.95 (dd, $^3J_{3,4}$ = 10.0 Hz, 3 H, 3 H-3), 5.73 (dd, $^3J_{2,3}$ = 3.3 Hz, 3 H, 3 H-2), 5.17 (d, $^3J_{1,2}$ = 1.9 Hz, 3 H, 3 H-1), 4.72 (dd, $^2J_{6,6'}$ = 13.0 Hz, 3 H, 3 H-6), 4.53–4.46 (m, $^3J_{5,6}$ = 3.5 Hz, 6 H, 3 H-5, 3 H-6′), 3.97–3.91 (m, 3 H, 3 manO–C*H*H), 3.83–3.68 (m, 11 H, 3 manOCH*H*, 3 manOCH$_2$C*H*$_2$, CH$_2$NCS), 3.60 [d, 6 H, C(CH$_2$O)$_3$] ppm. ^{13}C NMR (125.75 MHz, CDCl$_3$): δ = 166.12, 165.48, 165.38, 165.29 (12 C=O), 133.39, 133.36, 133.06, 133.02 [OC(O)C^{Bz}], 129.90, 129.83, 129.74, 129.73, 129.36, 129.14, 129.00, 128.53, 128.44, 128.42, 128.25 (C^{Bz}), 97.66 (3 C-1), 70.60 (3 manO–CH$_2$*C*H$_2$), 70.47 (3 C-2), 70.08 (3 C-3), 69.70 [C(*C*H$_2$O)$_3$], 68.81 (3 C-5), 67.12 (3 manO–CH$_2$), 66.98 (3 C-4), 62.87 (3 C-6), C$_q$ (46.03), 45.78 (*C*H$_2$NCS) ppm. MALDI-TOF MS (norharmane): *m*/*z* = 2068.0 [M – Na]$^+$ (calcd. *m*/*z* = 2068.06) for $C_{117}H_{108}N_2NaO_{33}S$.

***N*-{3-[2′-(2′′,3′′,4′′,6′′-Tetra-*O*-benzoyl-α-D-mannopyranosyloxy)ethoxy]-2,2-bis[2′-(2′′,3′′,4′′,6′′-tetra-*O*-benzoyl-α-D-mannopyranosyloxy)ethoxymethyl]propyl}-*N*′-(2′′′-azipropyl)thiourea (26):** The isothiocyanate **25** (318 mg, 0.16 mmol) was dissolved in dry dichloromethane (3 mL). DIPEA (50 μL) and a solution of the diazirine **4** (13 mg, 0.16 mmol) in dichloromethane (0.5 mL) were added and the reaction mixture was stirred at 30 °C for 8 h. The solvent was removed in vacuo and purification of the residue by column chromatography (cyclohexane/ethyl acetate, 2:1) yielded **26** (265 mg, 0.12 mmol, 80%) as a colorless solid. $[\alpha]_D^{20} = -35.0$ (*c* = 0.16, CHCl$_3$). ^{1}H NMR (500 MHz, CDCl$_3$): δ = 8.09, 8.02, 7.93, 7.82 (each dd, each 6 H, H^{Bz}), 7.58–7.53 (m, 6 H, H^{Bz}), 7.47–7.28 (m, 24 H, H^{Bz}), 7.25–7.21 (t, 6 H, H^{Bz}), 6.14 (t, $^3J_{4,5}$ = 10.1 Hz, 3 H, H-4), 5.94 (dd, $^3J_{3,4}$ = 10.1 Hz, 3 H, H-3), 5.73 (dd, $^3J_{2,3}$ = 3.2 Hz, 3 H, H-2), 5.20 (d, $^3J_{1,2}$ = 1.6 Hz, 3 H, H-1), 4.73 (dd, $^3J_{5,6'}$ = 2.5 Hz, 3 H, H-6′), 4.54–4.47 (m, $^3J_{5,6}$ = 4.1, $^2J_{6,6'}$ = 12.1 Hz, 6 H, H-5, H-6), 3.99–3.92 (m, 3 H, manOC*H*HCH$_2$), 3.85–3.61 [m, 19 H, manOCH*H*CH$_2$, manOCH$_2$C*H*$_2$, C*H*$_2$NHC(S), C(CH$_2$N)], 3.61 [s, 6 H, C(CH$_2$O)$_3$], 0.99 [s, 3 H, C(NN)CH$_3$] ppm. ^{13}C NMR (125.75 MHz, CDCl$_3$): δ = 166.17, 165.53, 165.49, 165.42 (C=O), 133.42, 133.40, 133.15, 133.06 [OC(O)C^{Bz}], 129.89, 129.85, 129.78, 129.74, 129.29, 129.08, 128.99, 128.56, 128.47, 128.30 (C^{Bz}), 97.67 (C-1), 70.62 (manOCH$_2$*C*H$_2$), 70.60 C(*C*H$_2$O)$_3$) , 70.52 (C-2), 70.16 (C-3), 68.90 (C-5), 67.28 (manO*C*H$_2$CH$_2$), 66.89 (C-4), 62.87 (C-6), 43.47 (C$_q$) ppm. MALDI-TOF MS (norharmane): *m*/*z* = 2102.1 [M – N$_2$]$^+$ (calcd. *m*/*z* = 2102.17) for $C_{117}H_{108}N_2O_{33}S$.

***N*-{3-[2′-(α-D-Mannopyranosyloxy)ethoxy]-2,2-bis[2′-(α-D-mannopyranosyloxy)ethoxymethyl]propyl}-*N*′-(2′′-azipropyl)thiourea (27):** The protected cluster **26** (225 mg, 0.11 mmol) was dissolved in a 1:1 mixture of dry MeOH and THF (2 mL) and mixed with sodium methoxide (1 M in MeOH, 126 μL). The solution was stirred for 1 h at room temp., then neutralised with Amberlite ion-exchange resin IR 120. After filtration and removal of the solvent the residue was purified by column chromatography (RP-18, water/MeOH, 5:1, then lyophilisation) to yield **27** (51 mg, 0.06 mmol, 55%) as a colorless foam. $[\alpha]_D^{20} = 21.3$ (*c* = 0.70, H$_2$O). ^{1}H NMR (500 MHz, D$_2$O): δ = 4.90 (d, 3 H, 3 H-1), 3.98–3.94 (m, 3 H, 3 H-2), 3.90–3.61 [m, 31 H, 3 H-3, 3 H-4, 3 H-5, 3 H-6, 3 H-6′, 3 manO–C*H*$_2$C*H*$_2$, C*H*$_2$NHC(S)], 3.56–3.46 [m, 6 H, C(CH$_2$O)$_3$], 3.34 [s, 2 H, C(CH$_2$N)], 1.08 [s, 3 H, C(NN)CH$_3$] ppm. ^{13}C NMR (125.75 MHz, D$_2$O): δ = 102.23 (3 C-1), 75.14 (3 C-4), 73.02 (3 C-3), 72.74 (3 manO–CH$_2$*C*H$_2$), 72.44 (3 C-2), 69.12 (3 C-5), 68.63 [C(*C*H$_2$O)$_3$], 64.87 (3 manO–*C*H$_2$), 51.26 [*C*H$_2$NHC(S)], 46.79 (C$_q$), 26.25 [C(NN)], 19.37 [C(NN)*C*H$_3$] ppm. MALDI-TOF MS (DHB): *m*/*z* = 875.5 [M + Na – N$_2$]$^+$ (calcd. *m*/*z* = 875.33) for $C_{33}H_{60}N_2NaO_{21}S$. ESI MS: *m*/*z* = 903.3358 [M + Na]$^+$ (calcd. *m*/*z* = 903.3363) for $C_{33}H_{60}N_4NaO_{21}S$.

1,9-Bis(2′,3′:4′,6′-diisopropylidene-α-D-mannopyranosyloxy)-3,7-dioxanonan-5-one (29): The alkene **28** (1.44 g 2.2 mmol) was dissolved in a 1:1 mixture of dry dichloromethane and methanol (20 mL) and cooled to –78 °C. Oxygen was passed through the reaction mixture for 5 min, then ozone for 20 min, then again oxygen for 5 min and finally argon for 5 min. Then triphenylphosphane (2.22 g, 8.5 mmol) was added to the cold solution which was then stirred for 2 h at room temp. After evaporation of the solvent in vacuo the residue was purified by flash chromatography (cyclohexane/ethyl acetate, 2:1) to yield **29** (1.33 g, 2.0 mmol, 92%) as an amorphous colorless solid. $[\alpha]_D^{20} = +6.8$ (*c* = 1.25, CHCl$_3$). ^{1}H NMR (500 MHz, CDCl$_3$): δ = 5.07 (s, 2 H, 2 H-1), 4.31 (d, 8 H, 4 H$_{c,c'}$), 4.22 (d, $^3J_{2,3}$ = 5.7 Hz, 2 H, 2 H-2), 4.16 (dd, $^3J_{3,4}$ = 7.9 Hz, 2 H, 2 H-3), 3.89–3.82 (m, 4 H, 2 H-6, 2 H$_a$), 3.79–3.64 (m, 10 H, 2 H-4, 2 H-6′, $^2J_{6,6'}$ = 10.8 Hz, 4 H$_{b,b'}$, 2 H$_{a'}$), 3.59 (dt, $^3J_{5,6}$ = 5.6 Hz, 2 H, 2 H-5), 1.55, 1.52, 1.42, 1.35 (each s, each 6 H, 8 *i*Pr-CH$_3$) ppm. ^{13}C NMR (125.75, CDCl$_3$): δ = 205.59 (C=O), 109.46 (2 2,3-*i*Pr-C$_q$), 99.70 (2 4,6-*i*Pr-C$_q$), 97.92 (2 C-1), 75.94 (2 C-2), 75.01 (2 C$_c$), 74.80 (2 C-3), 72.68 (2 C-4), 70.73 (2 C$_b$), 66.56 (2 C$_a$), 62.02 (2 C-6), 61.45 (2 C-5), 29.04, 28.15, 26.14, 18.77 (8 *i*Pr-CH$_3$) ppm. MALDI-TOF MS (norharmane): *m*/*z* = 685.75 [M + Na]$^+$ (calcd. *m*/*z* = 685.30) for $C_{31}H_{50}NaO_{15}$, *m*/*z* = 701.71 [M – K]$^+$ (calcd. *m*/*z* = 701.25) for $C_{31}H_{50}KO_{15}$.

1,9-Bis(2′,3′:4′,6′-diisopropylidene-α-D-mannopyranosyloxy)-5-azi-3,7-dioxanonane (30): The ketone **29** (810 mg, 1.22 mmol) was dissolved in a 1:2 mixture of dry dichloromethane and methanol (5 mL). After cooling of the solution to –50 °C gaseous NH$_3$ was added until a volume of approx. 80 mL had condensed. The solu-

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tion was stirred for 4 h at –20 °C. Then hydroxylamine-*O*-sulfonic acid (152 mg, 1.24 mmol), dissolved in dry methanol (2 mL), was added slowly at –50 °C. Cooling was stopped after 1 h and the solution was left standing overnight at room temp. The solution was filtered and concentrated. The remaining residue was dissolved in methanol (20 mL) and triethylamine (10 mL). This solution was titrated with a 10% methanolic iodine solution until the colour remained yellow. This solution was again concentrated and the residue dissolved in diethyl ether and water. The aqueous phase was extracted twice with diethyl ether and the combined organic phases were washed once with aqueous sodium thiosulfate solution and once with water. After drying with sodium sulfate and filtration purification by column chromatography (cyclohexane/ethyl acetate, 5:2) the diazirine **30** was obtained (404 mg, 0.60 mmol, 49%) as a colorless solid. $[\alpha]_D^{20}$ = +5.7 (*c* = 1.15, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.97 (d, $^3J_{1,2} \approx 0$ Hz, 2 H, 2 H-1), 4.13 (dd≈d, $^3J_{2,3}$ = 5.7 Hz, 2 H, 2 H-2), 4.08 (dd, $^3J_{2,3}$ = 7.9 Hz, 2 H, 2 H-3), 3.81 (dd, $^2J_{5,6}$ = 5.6 Hz, 2 H, 2 H-6), 3.74–3.64 (m, 6 H, 2 H-4, 2 H-6′, $^3J_{2,3}$ = 10.8 Hz, 2 H_a), 3.57–3.48 (m, 8 H, 2 H-5, 2 $H_{c,c'}$, 2 $H_{a'}$), 3.39 (d, 2 H, 2 H_b), 3.33 (d, 2 H, 2 $H_{b'}$), 1.48, 1.45, 1.35, 1.28 (each s, each 6 H, 8 *i*Pr-CH_3) ppm. ^{13}C NMR (125.75, $CDCl_3$): δ = 109.44 (2 2,3-*i*Pr-C_q), 99.69 (2 4,6-*i*Pr-C_q), 97.80 (2 C-1), 75.96 (2 C-2), 74.81 (2 C-3), 72.70 (2 C-4), 70.29 (2 C_b), 70.01 (2 C_c), 66.47 (2 C_a), 62.03 (2 C-6), 61.39 (2 C-5), 29.05, 28.17, 26.15, 18.78 (8 *i*Pr-CH_3), 27.38 [C(NN)] ppm. MALDI-TOF MS (norharmane): *m*/*z* = 669.79 $[M + Na - N_2]^+$ (calcd. *m*/*z* = 669.31) for $C_{31}H_{50}NaO_{15}$.

1,9-Bis(α-D-mannopyranosyloxy)-5-azi-3,7-dioxanonane (31): The protected compound **30** (187 mg, 0.28 mmol) was dissolved in a 9:1 mixture of TFA and water (10 mL). After 1 min the solution was concentrated in vacuo at room temp. The residue was codistilled twice with toluene and after column chromatography (ethyl acetate/methanol, 1:2) the title compound (133 mg, 0.26 mg, 93%) was delivered as a colorless solid. $[\alpha]_D^{20}$ = +57.1 (*c* = 0.95, MeOH). ^{1}H NMR (500 MHz, CD_3OD): δ = 4.84 (d, $^3J_{1,2}$ = 1.4 Hz, 2 H, H-1), 3.90–3.82 (m, $^3J_{2,3}$ = 3.3, $^3J_{5,6'}$ = 2.4 Hz, 6 H, H-2, H-6′, H_a), 3.79–3.72 (m, $^3J_{5,6}$ = 5.9, $^2J_{6,6'}$ = 11.7 Hz, 4 H, H-3, H-6), 3.69–3.57 (m, 10 H, H-4, H-5, $H_{a'}$, $H_{c,c'}$), 3.47 (d, 4 H, $H_{b,b'}$) ppm. ^{13}C NMR (125.75, CD_3OD): δ = 101.70 (C-1), 74.60 (C-5), 72.55 (C-3), 72.09 (C-2), 71.33 (C_c), 71.13 (C_b), 68.57 (C-4), 67.63 (C_a), 62.88 (C-6), 28.69 [C(NN)] ppm. MALDI-TOF MS (DHB): *m*/*z* = 509.54 $[M + Na - N_2]^+$ (calcd. *m*/*z* = 509.19) for $C_{19}H_{34}NaO_{14}$, 537.52 $[M + Na]^+$ (calcd. *m*/*z* = 537.19) for $C_{19}H_{34}N_2NaO_{14}$. ESI MS: *m*/*z* = 537.2150 $[M + Na]^+$ (calcd. *m*/*z* = 537.1902) for $C_{19}H_{34}N_2NaO_{14}$.

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[1] a) Y. Hatanaka, H. Nakayama, Y. Kanaoka, *Rev. Heteroat. Chem.* **1996**, *14*, 213–243; b) Y. Hatanaka, Y. Kanaoka, *Heterocycles* **1998**, *47*, 625–632; c) G. Dormán, G. D. Prestwich, *Trends Biotechnol.* **2000**, *18*, 64–77; d) G. Dormán, *Top. Curr. Chem.* **2000**, *211*, 169–225; e) F. Kotzyba-Hilbert, I. Kapfer, M. Goeldner, *Angew. Chem.* **1995**, *107*, 1391–1408; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1296–1312.
[2] S. A. Fleming, *Tetrahedron* **1995**, *51*, 12479–12520.
[3] A. Blencowe, W. Hayes, *Phys. Rev. E: Stat., Nonlinear, Soft Matter* **2005**, *1*, 178–205.
[4] a) J. Yang, A. E. Clark, I. J. Kozka, S. W. Cushman, G. D. Holman, *J. Biol. Chem.* **1992**, *267*, 10393–10399; b) Y. Hatanaka, Y. Sadakane, *Curr. Top. Med. Chem.* **2002**, *2*, 271–288.
[5] J. Lehmann, L. Ziser, *Carbohydr. Res.* **1992**, *225*, 83–97.
[6] M. Vetsch, C. Puorger, T. Spirig, U. Grauschopf, E.-U. Weber-Ban, R. Glockshuber, *Nature* **2004**, *431*, 329–332.
[7] a) D. Coudhury, A. Thompson, V. Stojanoff, S. Langermann, J. Pickner, S. J. Hultgren, S. D. Knight, *Science* **1999**, *285*, 1061–1066; b) C. S. Hung, J. Bouckaert, D. Hung, J. Pinkner, C. Widberg, A. DeFusco, C. G. Auguste, R. Strouse, S. Langermann, G. Waksman, S. J. Hultgren, *Mol. Microbiol.* **2002**, *44*, 903–915; c) J. Bouckaert, J. Berglund, M. Schembri, E. D. Genst, L. Cools, M. Wuhrer, C.-S. Hung, J. Pinkner, R. Slättegård, A. Zavialov, D. Choudhury, S. Langermann, S. J. Hultgren, L. Wyns, P. Klemm, S. Oscarson, S. D. Knight, H. D. Greve, *Mol. Microbiol.* **2005**, *55*, 441–455.
[8] a) F. G. Sauer, M. Barnhart, D. Choudhury, S. D. Knight, S. D. Wacksmann, S. J. Hultgren, *Curr. Opin. Struct. Biol.* **2000**, *10*, 548–556; b) S. D. Knight, J. Berglund, D. Choudhury, *Curr. Opin. Chem. Biol.* **2000**, *4*, 653–660.
[9] a) Th. K. Lindhorst, S. Kötter, U. Krallmann-Wenzel, S. Ehlers, *J. Chem. Soc., Perkin Trans. 1* **2001**, 823–831; b) Th. K. Lindhorst, *Top. Curr. Chem.* **2002**, *218*, 201–235; c) R. Autar, A. S. Khan, M. Schad, J. Hacker, R. M. J. Liskamp, R. J. Pieters, *ChemBioChem* **2003**, *4*, 1317–1325; d) N. Nagahori, R. T. Lee, S.-I. Nishimura, D. Pagé, R. Roy, Y. C. Lee, *ChemBioChem* **2002**, *3*, 836–844.
[10] M. Walter, Th. K. Lindhorst, *Synthesis*, in press.
[11] L. Vannucci, A. Fišerová, K. Sadalapure, Th. K. Lindhorst, M. Kuldová, P. Rossmann, O. Horváth, V. Křen, P. Krist, K. Bezouška, M. Luptovcová, F. Mosca, M. Pospíšil, *Int. J. Oncology* **2003**, *23*, 285–296.
[12] G. Zemplén, E. Pascu, *Ber.* **1929**, *62*, 1613–1614.
[13] R. R. Schmidt, W. Kinzy, *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21–123.
[14] F. Bien, T. Ziegler, *Tetrahedron: Asymmetry* **1998**, *9*, 781–790.
[15] B. Schönecker, K. Ponsold, *J. Prakt. Chem.* **1976**, *318*, 483–488.
[16] A. E. Clark, G. D. Holman, *Biochem. J.* **1990**, *269*, 615–622.
[17] M. M. K. Boysen, K. Elsner, O. Sperling, Th. K. Lindhorst, *Eur. J. Org. Chem.* **2003**, 4376–4386.
[18] a) R. F. R. Church, M. J. Weiss, *J. Org. Chem.* **1970**, *35*, 2465–2471; b) J. Lehmann, U. P. Weitzel, *Carbohydr. Res.* **1996**, *294*, 65–94.

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