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Chemoenzymatic Synthesis of Functional Sialyl Lewis^X Mimetics with a Heteroaromatic Core

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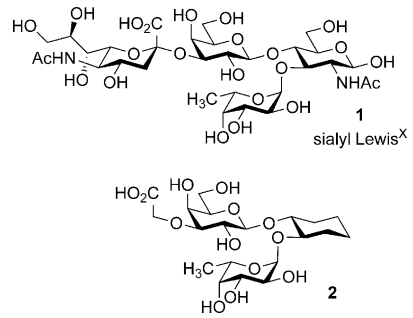
In memory of Alan R. Katritzky

Abstract: Functional mimetics of the sialyl Lewis^X tetrasaccharide were prepared by the enzymatic sialylation of a 1,3-diglycosylated indole and a glycosyl azide, which was subsequently transformed into a 1,4-diglycosylated 1,2,3-triazole, by using the *trans*-sialidase of *Trypanosoma cruzi*. These compounds inhibited the binding of E-, L-, and P-selectin-coated nanoparticles to polyacrylamide-bound sialyl-Lewis^X-containing neighboring sulfated tyrosine residues (sTyr/sLe^X-PAA) at low or sub-millimolar concentrations. Except for E-selectin, the mimetics showed higher activities than the natural tetrasaccharide.

Keywords: cell adhesion · enzyme catalysis · heterocycles · oligosaccharides · saccharide mimetics

Introduction

Cell–cell interactions are a prerequisite for the functioning of multicellular organisms. Besides protein–protein interactions, the recognition of glycostructures on the cell surface by cognate (glyco)protein receptors—lectins—is one of the two major mechanisms of cell adhesion.[1] In humans and animals, the attractive interactions between leukocytes, which patrol the bloodstream, and the activated endothelium of blood vessels, which indicates tissue inflammation, play a key role in the inflammatory cascade, which is an important part of the immune response against pathogenic microbes. Recognition of the tetrasaccharide sialyl Lewis^X (sLe^X, α-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-D-Glc*p*NAc, **1**; Scheme 1) by endothelial (E-), platelet (P-), and lymphocyte (L-)selectin constitutes the initial step in this physiological process.[2]



Scheme 1. Structures of the sialyl Lewis^X tetrasaccharide and mimetic **2**.

The excessive accumulation of leukocytes in an inflamed tissue, initiated by exaggregated leukocyte adhesion to activated vascular endothelia, is thought to be the main cause of secondary tissue destruction in various chronic diseases, such as asthma,[3] psoriasis,[4] and rheumatoid arthritis.[5] Selectin-based cell adhesion is also involved in pathomechanisms, such as tumor metastasis[6] or the reperfusion syndrome,[7] which is observed upon restoring circulation after a period of ischemia in a diverse range of tissues and can contribute to complications in the transplantation of organs. In all of these cases, inhibition of the sLe^X–selectin interactions is desirable. To achieve this inhibition, numerous mimetics of the sLe^X molecule have been synthesized that are

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capable of competing with their natural antetype for the binding site in the C-type lectin domain of the selectins.[7a,8] These mimetics belong to various structural classes, ranging from all-carbohydrate compounds to peptides and N-heterocycles.[9]

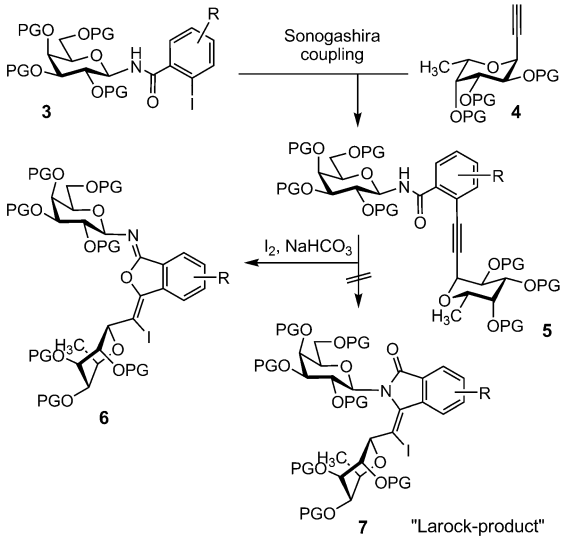
The crystal structures of complexes of sLe^X with E- and P-selectin show that, among all four saccharide units, the GlcNAc residue has the weakest interactions with both receptors.[2] In previously reported mimetics, the GlcNAc residue has been substituted by cyclic and acyclic 1,2-diols to imitate the relative arrangement of the 3-sialylated galactose residue and the fucose moiety in sLe^X.[8] In particular, (1*R*,2*R*)-cyclohexanediol was found to be a well-suited GlcNAc surrogate (see mimetic **2**; Scheme 1), whereas the use of more flexible or improperly aligning spacer units resulted in a loss of affinity towards E-selectin.[10] We wanted to investigate the possibility of replacing GlcNAc by a rigid heterocycle, which ideally should: 1) simplify the preparation of the respective mimetic; 2) permit the late-stage variation of the fucose moiety; and 3) use N- or C-glycosidic bonds to attach the pending hexose units to prevent enzymatic cleavage, whilst 4) retaining or even improving the affinity of the native tetrasaccharide.

Results and Discussion

Attempts to bring the galactose and fucose moieties into similar spatial proximity as in sLe^X by preparing diglycosylacetylenes and subjecting them to 1,3-dipolar cycloaddition or by transforming them into 2,3-diglycosylquinoxalines through oxidation into 1,2-diones and subsequent reaction with *o*-phenylenediamine met with little success. In contrast, C,N-diglycosylated *o*-alkynylbenzamides of type **5** could be readily prepared from glycosylamines by amide formation with 2-iodobenzoyl chloride; subsequent Sonogashira reaction with glycosylacetylenes to provide the starting materials for a Larock iodocyclization has been reported to furnish isoindolinones of type **7** (Scheme 2).[11]

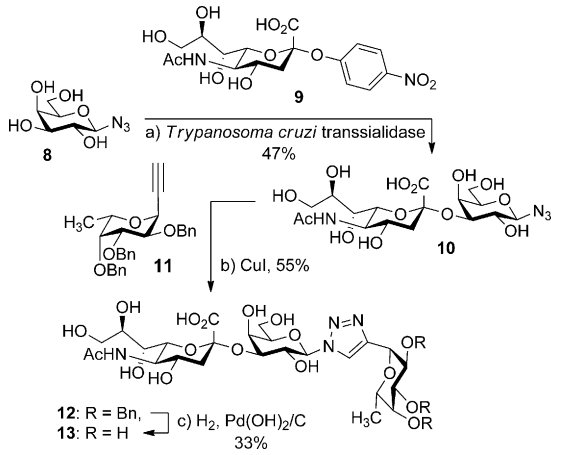
Although the reaction products exhibited spectroscopic properties that were very similar to literature data, the structural assignment of the known iodocyclization products was found to have been wrong and that isobenzofurans of type **6** were formed instead.[12] These latter products should not only provide a less-suitable spatial arrangement of the glycosyl moieties, but their structure also explains the observed instability towards the conditions for protecting-group removal.

The copper- (CuAAC)[13] or ruthenium-catalyzed (RuAAC)[14] 1,3-dipolar cycloaddition[15] of glycosylazides to glycosylacetylenes provided the expected 1,4- and 1,5-diglycosylated 1,2,3-triazoles, respectively, in appreciable yields.[11a] In particular, the CuAAC reaction was highly reliable and largely insensitive towards steric hindrance. Moreover, both types of triazoles were stable towards the hydrogenolytic removal of benzyl protecting groups on the glycosylacetylene moiety. Selective enzymatic sialylation of the 3-



Scheme 2. Diglycosylated heterocycles as potential core structures.

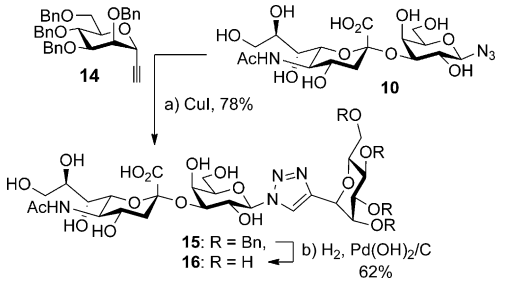
OH group of the galactopyranose unit was employed to transform β-D-galactopyranosylazide (**8**) into disaccharide azide **10**. This reaction uses the readily available *p*-nitrophenylsialoside **9** as the sialyl donor and is catalyzed by the *trans*-sialidase of *Trypanosoma cruzi*, the causal agent of Chagas disease (Scheme 3).[16] Subsequent CuAAC reaction



Scheme 3. Chemoenzymatic synthesis of mimetic **13**.

with fucosylacetylene **11** furnished C-fucoside **12**, which was subsequently debenzylated to yield mimetic **13** (Scheme 3).

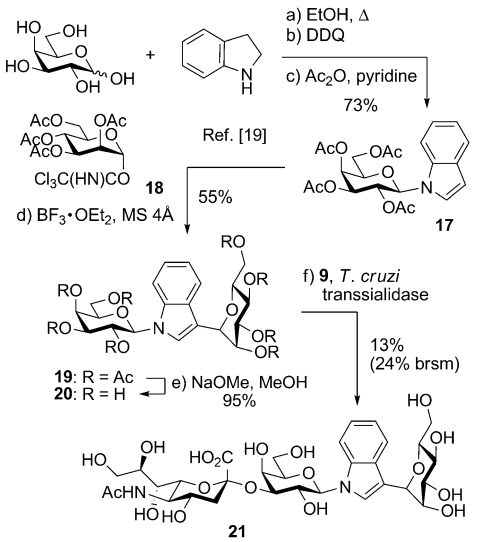
The specific role of the α-L-fucopyranosyl unit within the sLe^X tetrasaccharide is the complexation of a selectin-bound Ca^{2+} ion by the hydroxy groups at the 2-, 3-, and 4-positions.[2,17] α-D-Mannopyranosides present the same spatial arrangement of their three secondary alcohol functions with the axial 2-OH-group of mannose being equivalent to the 4-OH-group of fucose. Based on this analogy, various functional mannose-derived sLe^X mimetics were prepared.[18] To test whether the activity of mimetic **13** could be retained or



Scheme 4. Synthesis of C-mannoside **16**.

even improved in a similar fashion, the mannose analogue of compound **13**, C-mannoside **16**, was prepared accordingly from mannosylacetylene **14** (Scheme 4).

1,3-Diglycosylindoles are another class of potential trisaccharide mimetics and present the same relative arrangement of the two pendant glycosyl moieties as in 1,4-diglycosyl-1,2,3-triazoles. Although the installation of 1,3- and 2,3-diglycosylation patterns on the indole core has already been established,[19] the former compound class is particularly attractive, owing to the possibility of introducing both carbohydrate substituents in a straightforward sequential manner. The result of scaffold hopping from 1,2,3-triazole to indole on mimetic **16** is C-mannoside **21**, which could be prepared by the reaction of D-galactose with indoline and subsequent dehydrogenation of the resulting β-N-galactoside with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ),[20] followed by O-acetylation to afford protected 1-glycosylindole **17**. α-Selective C-glycosylation at the 3-position with peracetylated mannosyl trichloroacetimidate **18** produced 1,3-diglycosylindole **19**, which was deacetylated under Zemplén conditions to give trisaccharide mimetic **20**. The enzymatic sialylation reaction proceeded with complete regioselectivity and furnished sLe^X mimetic **21** as the sole product in 13% yield (24% based on recovered starting materials; Scheme 5).



Scheme 5. Chemoenzymatic synthesis of mimetic **21**.

Evaluation of Selectin Inhibition

The binding of the prepared sLe^X mimetics to the selectins was studied by using a competitive inhibition assay. In this assay, potential inhibitors compete with the binding of L-, P-, or E-selectin to the synthetic sulfo-tyrosine/sLe^X biligand under flow conditions.[21] Mimetics **13**, **16**, and **21** were compared to the performance of the sLe^X tetrasaccharide in this assay (Figure 1). Binding of L- and P-selectin is not inhibited by the natural sLe^X ligand within the tested concentration range since additional binding sites on these selectins

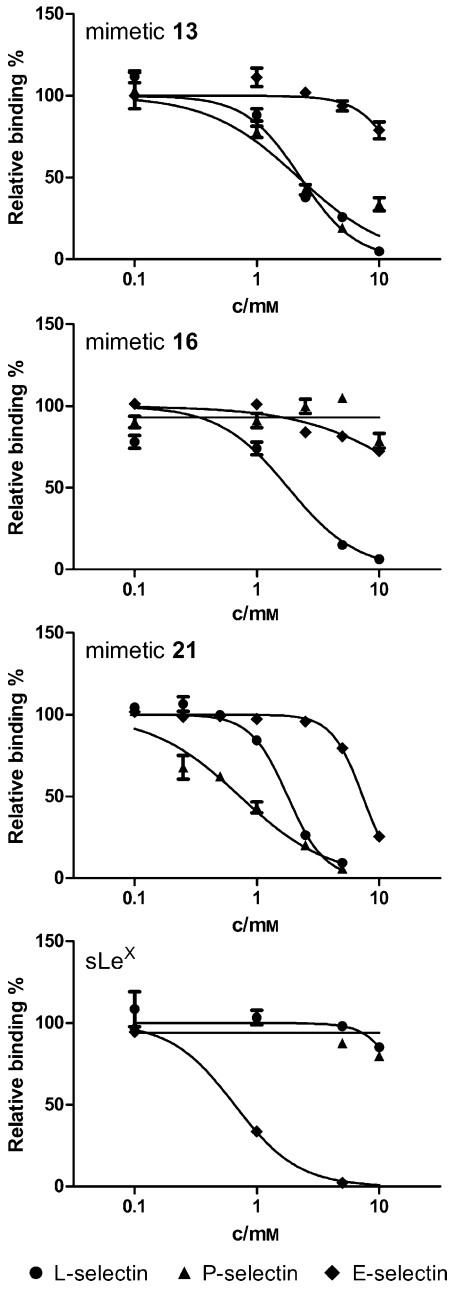


Figure 1. Performance of mimetics **13**, **16**, **21**, and sLe^X in a competitive selectin-inhibition assay, expressed as a plot of relative binding versus concentration.

Table 1. IC_{50} values [mM] of the sLe^X mimetics, as calculated from the dose-response curves.

Compound	IC_{50} value		
	L-selectin	P-selectin	E-selectin
13	2.3	2.2	–
16	1.8	–	–
21	1.8	0.7	7.4
sLe^X	–	–	0.7

need to be addressed. However, E-selectin is inhibited, with an IC_{50} value of about 0.7 mM (Table 1). In contrast, mimetic **13** shows inhibition of L- and P-selectin, with IC_{50} values of 2.2–2.3 mM, but no significant inhibition of E-selectin binding within the tested concentration range. Mimetic **16** only shows inhibition of L-selectin, with IC_{50} values within the same concentration range as for mimetics **13** and **21**. Indole-based mimetic **21** can inhibit all three selectins, with IC_{50} values from 0.7 mM (P-selectin) to 7.4 mM (E-selectin), and is as effective for L-selectin inhibition as the other two mimetics.

Thus, the mimetics tested show selectivity towards the three selectins: Whereas sLe^X is the best inhibitor of E-selectin and does not affect the other selectins within the tested concentration range, compound **21** is the best inhibitor of P-selectin and shows lower potency towards L- and E-selectin. Compound **13** inhibits P- and L-selectin in a similar manner and compound **16** shows a clear preference for L-selectin.

Conclusions

In summary, three heteroarene-based mimetics of sialyl $Lewis^X$ were synthesized by using chemoenzymatic methods. All three compounds were found to be better inhibitors than the native tetrasaccharide for at least one of the three selectins, with E-selectin the weakest binding partner in all cases. Whereas triazole-based C-mannoside **16** only showed appreciable binding to L-selectin, the analogous C-fucoside (**13**) inhibited P- and L-selectin binding with similar potency. The most-active compound was indole-based mimetic **21**, which affected all three selectins. Mimetic **21** showed a clear preference for P- over L-selectin and had less effect on E-selectin binding, thereby resulting in an IC_{50} value about one order of magnitude higher than that of sLe^X. However, the binding of L- and P-selectin was even more effectively inhibited than by the natural tetrasaccharide.

Interestingly, scaffold hopping from 1,2,3-triazole to indole on mimetic **16** generated high affinity for P-selectin whilst retaining the same activity for L-selectin. These results demonstrate that functional sLe^X mimetics can be obtained without any particular relative preorganization of the 3-sialylgalactose unit with the fucose moiety or a suitable substitute through an *exo*-anomeric effect. Substitution of the GlcNAc portion of sLe^X, the impact of which on the overall conformation of the tetrasaccharide has been thoroughly investigated,[22] by two simple heteroarenes even leads to compounds with improved affinity for P- and/or L-selectin without the need to address distal binding pockets.[23] Although the metabolic stability of the as-prepared mimetics has not yet been tested, complete inertness of the two modified glycosidic bonds against degrading glycosidases is to be expected because the essential structural features for the action of these enzymes are missing. It is likely that heteroarene-based glycomimetics can also be used to target further carbohydrate-binding proteins, which may constitute an interesting opportunity for drug development.

Experimental Section

For materials and methods, see the Supporting Information.

Expression and Purification of Trans-sialidase

T. cruzi trans-sialidase,[16c,25] which contained a hexahistidine tag, was expressed in *E. coli* M15 pRep4 cells (Qiagen) in terrific broth medium at 18 °C for 16 h and 120 rpm. The cells were harvested, re-suspended in 50 mM sodium phosphate buffer (pH 8.0) that contained 0.3 M NaCl and 0.05% Lubrol, and sonicated. After centrifugation, the supernatant was applied onto a His-Trap matrix (GE Healthcare) and washed with 50 mM sodium phosphate buffer (pH 8.0) that contained 0.3 M NaCl. *Trans*-sialidase was eluted with 50 mM sodium phosphate buffer (pH 8.0) that contained 250 mM imidazole with a purity of 95% and dialyzed against 20 mM Tris buffer (pH 7.6) that contained 30 mM NaCl.

Competitive Selectin-Inhibition Assay

Sialyl $Lewis^X$ mimetics were tested for their ability to inhibit selectin-mediated binding by using a competitive SPR assay, as described by Enders et al.[21] Briefly, recombinant selectin Fc-chimeras (R&D Systems) were coupled to gold nanoparticles that were coated with protein A (15 nm average, Aurion) in running buffer (20 mM HEPES pH 7,4; 150 mM NaCl, 1 mM $CaCl_2$) and passed over the surface of a sensor chip of a BIAcore X device (GE Healthcare). The sensor-chip surface was divided into two flow cells: a measurement cell, which was functionalized with sLe^X and sulfated tyrosine coupled to a polyacrylamide backbone, and a reference cell, which displays the non-binding LacNAc polymer in a similar manner. Both conjugates were immobilized onto the streptavidin sensor chip by using biotin. The unspecific binding signal from the reference cell was subtracted during the measurements. An aliquot (35 μL) of the selectin-coupled gold particles with the buffer or inhibitors was injected at a flow rate of 20 $\mu L\,min^{-1}$; the dissociation phase was 120 s. The data points were collected by using the difference of the binding signal immediately before injection (baseline) and at the end of the dissociation phase. All of the measurements were performed at least three times and averaged. The inhibitors were incubated for 18 min with the selectin gold particles prior to injection. The resulting reduced binding signal was divided by the binding values without the inhibitor (100%) at each corresponding point, which was calculated by linear regression of the 100% values taken before and after each series of measurements. The data were plotted as the percentage relative binding versus the concentration of inhibitor. The IC_{50} values were calculated by using a dose-response curve fit (log inhibitor versus normalized response-variable slope) in GraphPad Prism 5.

*3-O-(5-Acetamido-3,5-didesoxy-D-glycero-α-D-galactonon-2-ulopyranosyl)-β-D-galactopyranosyl azide (**10**)*

The title compound was prepared according to a method reported by Neubacher et al.[16b] Azide **8**[26] (400 mg, 1.95 mmol) and 4-nitrophenyl-α-sialoside **9**[27] (492 mg, 1.10 mmol) were dissolved in Tris-HCl-buffer (8 mL, 100 mM, pH 7.9). The mixture was cooled to 13 °C by using a cryostat and an aqueous solution of recombinant *trans*-sialidase (4 mL, $c = 0.93\ mg\,mL^{-1}$) was added. The mixture was kept at that temperature for

3 days. The enzyme was denatured by the addition of EtOH (10 mL) and the mixture was centrifuged. The supernatant was removed and the solvent was evaporated by lyophilization. The lyophilisate was purified by flash chromatography on silica gel (cyclohexane/EtOAc, 3:1→EtOAc/MeOH, 10:1→MeOH). The product contained remaining TRIS-buffer (13 mol% as judged by NMR spectroscopy), which could neither be removed by the weakly acidic cation-exchange resin Amberlite IRC-86 nor by repeated chromatography. Yield: 257 mg (0.518 mmol, 47%); colorless solid; R_f=0.12 (BuOH/AcOH/H_2O, 5:2:2); m.p. 175.0–176.0 °C; $[\alpha]_D^{24}=\pm 0.0$ (c=0.40, CD_3OD); ^{1}H NMR (COSY, 400 MHz, CD_3OD): δ=4.49 (d, $^3J_{1,2}$=8.5 Hz, 1H; H-1^{gal}), 4.04 (dd, $^3J_{2,3}$=9.6 Hz, $^3J_{3,4}$=2.7 Hz, 1H; H-3^{gal}), 3.95 (d, $^3J_{3,4}$=2.7 Hz, 1H; H-4^{gal}), 3.86–3.59 (m, 9H; H-5^{gal}, H-6a/b^{gal}, H-4^{sia}, H-5^{sia}, H-6^{sia}, H-8^{sia}, H-9a/b^{sia}), 3.55 (pseudo-t, $^3J_{1,2}$=$^3J_{2,3}$=9.2 Hz, 1H; H-2^{gal}), 3.50 (m, 1H; H-7^{sia}), 2.84 (dd, ^2J=11.8 Hz, $^3J_{3,4}$=3.3 Hz, 1H; H-3a^{sia}), 2.01 (s, 3H; CH_3^{NHAc}), 1.76–1.70 ppm (m, 1H; H-3b^{sia}); ^{13}C NMR, HSQC (100.6 MHz, CD_3OD): δ=175.5 (2C; C=O^{sia}, C=O^{NHAc}), 101.1 (C-2^{sia}), 92.3 (C-1^{gal}), 77.7 (C-3^{gal}), 70.3 (C-2^{gal}), 70.0 (C-7^{sia}), 78.9, 74.9, 72.9, 69.3 (C-5^{gal}, C-4^{sia}, C-6^{sia}, C-8^{sia}), 68.9 (C-4^{gal}), 64.4 (C-6^{gal}), 62.7 (C-9^{sia}), 53.9 (C-5^{sia}), 42.0 (C-3^{sia}), 22.6 ppm (CH_3^{NHAc}); MS (ESI): m/z (%): 519.1 [M+Na]$^+$ (100); HRMS (ESI): m/z calcd for [$C_{17}H_{28}N_4O_{13}$+Na]$^+$: 519.1551 [M+Na]$^+$; found: 519.1546.

*1-(3-O-(5-Acetamido-3,5-didesoxy-*D*-glycero-α-*D*-galactonon-2-ulopyranosyl))-β-*D*-galactopyranosyl-4-(2,3,4-tri-O-benzyl-α-*L*-fucopyranosyl)-1 H-1,2,3-triazole (**12**)*

The title compound was prepared according to a method reported by Dondoni and Marra.[28] Fucosylacetylene **11**[29] (82 mg, 0.19 mmol) and azide **10** (91 mg, 0.18 mmol) were dissolved in dry DMF (4 mL). *N*,*N*-Diisopropylethylamine (DIPEA, 64 μL) and CuI (7 mg, 0.04 mmol, 20 mol%) were added in a countercurrent of argon gas and the mixture was stirred for 15 h at 70 °C. The solvent was removed in vacuo and the residue was co-evaporated three times with toluene (5 mL each). The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 4:1→2:1) to yield the title compound (94 mg, 0.10 mmol, 55% yield) as a light-yellow oil. $[\alpha]_D^{24}=-51.6$ (c=0.6, MeOH); ^{1}H NMR (COSY, 600 MHz, CD_3OD): δ=8.20 (s, 1H; H-5), 7.41–7.40 (m, 2H; Ph), 7.37–7.22 (m, 13H; Ph), 5.65 (d, $^3J_{1,2}$=8.9 Hz, 1H; H-1^{gal}), 5.24 (d, $^3J_{1,2}$=5.4 Hz, 1H; H-1^{fuc}), 4.85 (d, ^2J=11.2 Hz, 1H; CH_2–Ph), 4.79 (d, ^2J=11.5 Hz, 1H; CH_2–Ph), 4.77 (d, ^2J=11.5 Hz, 1H; CH_2–Ph), 4.64–4.58 (m, 3H; CH_2–Ph), 4.27 (dd, $^3J_{2,3}$=9.6 Hz, $^3J_{3,4}$=2.0 Hz, 1H; H-3^{fuc}), 4.21–4.18 (m, 2H; H-2^{gal}, H-2^{fuc}), 4.09 (d, $^3J_{3,4}$=2.9 Hz, 1H; H-4^{gal}), 4.06 (dd, $^3J_{2,3}$=8.9 Hz, $^3J_{3,4}$=2.9 Hz, 1H; H-3^{gal}), 3.89–3.70 (m, 9H; H-5^{gal}, H-6a^{gal}, H-4^{fuc}, H-5^{fuc}, H-5^{sia}, H-6^{sia}, H-8^{sia}, H-9a/b^{sia}), 3.63–3.60 (m, 2H; H-6b^{gal}, H-4^{sia}), 3.51 (d, ^3J=8.9 Hz, 1H; H-7^{sia}), 2.90 (dd, ^2J=13.1 Hz, $^3J_{3,4}$=3.5 Hz, 1H; H-3a^{sia}), 2.01 (s, 3H; CH_3^{NHAc}), 1.80–1.79 (m, 1H; H-3b^{sia}), 1.19 ppm (d, $^3J_{5,CH3}$=6.4 Hz, 3H; CH_3^{fuc}); ^{13}C NMR (HSQC, HMBC, 150.9 MHz, CD_3OD): δ=175.5 (2C; C=O^{sia}, C=O^{NHAc}), 140.1 (3C; C-4, 2×Cq–Ph), 139.6 (Cq–Ph), 129.4 (4C), 129.34 (2C), 129.27 (2C), 129.2 (2C), 129.0 (2C), 128.79, 128.77, 128.67 (Ph), 124.4 (2C; C-5, C-2^{sia}), 90.0 (C-1^{gal}), 79.9, 78.3, 72.9, 70.8, 69.4 (C-5^{gal}, C-4^{fuc}, C-5^{fuc}, C-6^{sia}, C-8^{sia}), 79.3 (C-3^{gal}), 78.0 (C-3^{fuc}), 76.9 (C-2^{fuc}), 75.5, 74.2, 73.2 (CH_2–Ph), 75.0 (C-4^{sia}), 70.0 (C-7^{sia}), 69.8 (C-1^{fuc}), 69.6 (C-2^{gal}), 69.2 (C-4^{gal}), 64.5 (C-6^{gal}), 62.7 (C-9^{sia}), 54.0 (C-5^{sia}), 42.0 (C-3^{sia}), 22.6 (CH_3^{NHAc}), 16.5 ppm (CH_3^{fuc}); MS (ESI): m/z (%): 939.5 [M+H]$^+$ (100); HRMS (ESI): m/z calcd for [$C_{46}H_{58}N_4O_{17}$+Na]$^+$: 961.3695 [M+Na]$^+$; found: 961.3692.

*1-(3-O-(5-Acetamido-3,5-didesoxy-d-glycero-α-d-galactonon-2-ulopyranosyl))-β-d-galactopyranosyl-4-(α-l-fucopyranosyl)-1 H-1,2,3-triazole (**13**)*

Triazole **12** (100 mg, 110 μmol) was dissolved in MeOH (5 mL). The solution was degassed by ultrasonication under an argon atmosphere before $Pd(OH)_2$ on charcoal (45 mg, 20 wt%) was added. After a second degassing cycle, the argon atmosphere was replaced with hydrogen (balloon). After stirring for 3 days at RT, another portion of the catalyst (45 mg) was added, the hydrogen pressure was increased to 3.5 bar (Parr bomb), and the suspension was stirred for a further 5 days at RT. The catalyst was removed by filtration over Celite and thoroughly washed with MeOH. The solvent was removed in vacuo and the crude product was purified by preparative HPLC (ACE-C_{18}-PFP) to yield the title compound (24 mg, 36 μmol, 33% yield) as a colorless oil. $[\alpha]_D^{24}=-39.1$ (c=0.5, D_2O); ^{1}H NMR (COSY, NOESY, ROESY, 600 MHz, D_2O): δ=8.36 (s, 1H; H-5), 5.78 (d, $^3J_{1,2}$=8.9 Hz, 1H; H-1^{gal}), 5.30 (d, $^3J_{1,2}$=6.6 Hz, 1H; H-1^{fuc}), 4.32 (dd, $^3J_{2,3}$=9.7 Hz, $^3J_{3,4}$=3.0 Hz, 1H; H-3^{gal}), 4.27 (pseudo-t, $^3J_{1,2}$=8.9 Hz, $^3J_{2,3}$=9.7 Hz, 1H; H-2^{gal}), 4.19 (dd, $^3J_{1,2}$=6.6 Hz, $^3J_{2,3}$=10.4 Hz, 1H; H-2^{fuc}), 4.08–4.05 (m, 2H; H-4^{gal}, H-3^{fuc}), 3.98 (t, $^3J_{5,6}$=6.1 Hz, 1H; H-5^{gal}), 3.89–3.80 (m, 5H; H-4^{fuc}, H-5^{fuc}, H-5^{sia}, H-6^{sia}, H-9a^{sia}), 3.73 (d, $^3J_{5,6}$=6.1 Hz, 2H; H-6^{gal}), 3.68–3.66 (m, 1H; H-4^{sia}), 3.61–3.58 (m, 2H, H-8^{sia}; H-9b^{sia}), 3.56 (dd, ^3J=9.1 Hz, ^3J=1.8 Hz, 1H; H-7^{sia}), 2.73 (dd, ^2J=12.4 Hz, $^3J_{3,4}$=4.8 Hz, 1H; H-3a^{sia}), 1.99 (s, 3H; CH_3^{NHAc}), 1.80 (pseudo-t, ^2J=12.4 Hz, $^3J_{3,4}$=12.1 Hz, 1H; H-3b^{sia}), 1.14 ppm (d, $^3J_{5,CH3}$=6.5 Hz, 3H; CH_3^{fuc}); ^{13}C NMR (HSQC, HMBC, 150.9 MHz, D_2O): δ=174.9 (C=O^{NHAc}), 173.8 (C=O^{sia}), 143.3 (C-4) 124.8 (C-5), 99.9 (C-2^{sia}), 87.6 (C-1^{gal}), 78.0 (C-5^{gal}), 75.7 (C-3^{gal}), 72.8 (C-8^{sia}), 71.7 (C-6^{sia}), 71.6 (C-4^{fuc}), 70.5 (C-1^{fuc}), 70.1 (C-3^{fuc}), 69.3 (C-5^{fuc}), 68.3 (C-4^{sia}), 68.0 (C-7^{sia}), 67.9 (C-2^{gal}), 67.6 (C-4^{gal}), 67.2 (C-2^{fuc}), 62.5 (C-9^{sia}), 60.8 (C-6^{gal}), 51.6 (C-5^{sia}), 39.4 (C-3^{sia}), 22.0 (CH_3^{NHAc}), 15.6 ppm (CH_3^{fuc}); MS (ESI): m/z (%): 691.3 [M+Na]$^+$ (100), 669.3 [M+H]$^+$ (89); HRMS (ESI): m/z calcd for [$C_{25}H_{40}N_4O_{17}$+Na]$^+$: 691.2286 [M+Na]$^+$; found: 691.2283; t_r=2.1 min (H_2O/MeCN, 98:2→98:2 (5 min)→80:20 (15 min); flow rate=38.00 mL min^{-1}).

*1-(3-O-(5-Acetamido-3,5-didesoxy-*D*-glycero-α-*D*-galactonon-2-ulopyranosyl))-β-*D*-galactopyranosyl-4-(2,3,4,6-tetra-O-benzyl-α-*D*-mannopyranosyl)-1 H-1,2,3-triazole (**15**)*

The title compound was prepared according to a procedure reported by Dondoni and Marra.[28] Mannosylacetylene **14**[29] (55.0 mg, 0.1 mmol) and azide **10** (50.0 mg, 0.10 mmol) were dissolved in dry DMF (2 mL). DIPEA (35 μL) and CuI (4.00 mg, 0.01 mmol, 10 mol%) were added in a countercurrent of argon gas and the mixture was stirred for 15 h at 70 °C. The solvent was removed in vacuo and the residue was co-evaporated three times with toluene (5 mL each). The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 2:0.2+0.1% AcOH→CH_2Cl_2/MeOH 1:1+0.1% AcOH) to yield the title compound (82.0 mg, 78.0 μmol, 78% yield) as a light-yellow oil. R_f=0.57 (CH_2Cl_2/MeOH 1:1+0.1% AcOH); $[\alpha]_D^{20}=+2.5$ (c=1.0, MeOH); ^{1}H NMR (COSY, 400 MHz, CD_3OD): δ=8.16 (s, 1H; H-5), 7.39–7.37 (m, 2H; Ph), 7.33–7.22 (m, 16H; Ph), 7.18–7.15 (m, 2H; Ph), 5.66 (d, $^3J_{1,2}$=9.2 Hz, 1H; H-1^{gal}), 5.30 (d, $^3J_{1,2}$=3.3 Hz, 1H; H-1^{man}), 4.75 (d, ^2J=11.0 Hz, 1H; CH_2–Ph), 4.70 (d, ^2J=12.3 Hz, 1H; CH_2–Ph), 4.69 (d, ^2J=11.6 Hz, 1H; CH_2–Ph), 4.61 (d, ^2J=11.6 Hz, 1H; CH_2–Ph), 4.59–4.52 (m, 2H; CH_2–Ph), 4.51–4.49 (m, 2H; CH_2–Ph), 4.43 (pseudo-t, $^3J_{1,2}$=$^3J_{2,3}$=3.3 Hz, 1H; H-2^{man}), 4.28 (dd, $^3J_{2,3}$=9.3 Hz, $^3J_{3,4}$=2.6 Hz, 1H; H-3^{gal}), 4.22 (pseudo-t, $^3J_{1,2}$=$^3J_{2,3}$=9.2 Hz, 1H; H-2^{gal}), 4.09 (d, $^3J_{3,4}$=2.6 Hz, 1H; H-4^{gal}), 3.99 (pseudo-t, $^3J_{3,4}$=$^3J_{4,5}$=8.0 Hz, 1H; H-4^{man}), 3.95 (dd, $^3J_{2,3}$=3.3 Hz, $^3J_{3,4}$=8.0 Hz, 1H; H-3^{man}), 3.86–3.59 (m, 12H; H-5^{gal}, H-6a/b^{gal}, H-5^{man}, H-6a/b^{man}, H-4^{sia}, H-5^{sia}, H-6^{sia}, H-8^{sia}, H-9a/b^{sia}), 3.52 (dd, ^3J=8.9 Hz, ^3J=1.5 Hz, 1H; H-7^{sia}), 2.89 (dd, ^2J=12.7 Hz, $^3J_{3,4}$=4.2 Hz, 1H; H-3a^{sia}), 2.03 (s, 3H; CH_3^{NHAc}), 1.82–1.76 ppm (m, 1H; H-3b^{sia}); ^{13}C NMR (HSQC, 100.6 MHz, CD_3OD): δ=175.5 (2C; C=O^{sia}, C=O^{NHAc}), 146.2 (C-4), 139.8, 139.7, 139.6, 139.5 (4×Cq–Ph), 129.43 (2C), 129.41 (4C), 129.33 (2C), 129.25 (2C), 129.2 (2C), 129.1 (2C), 129.0 (2C), 128.8 (2C), 128.7, 128.6 (Ph), 123.6 (2C; C-5, C-2^{sia}), 90.0 (C-1^{gal}), 79.9 (C-3^{man}), 77.9 (C-3^{gal}), 76.6 (C-2^{man}), 76.0 (C-4^{man}), 75.3, 74.3, 73.3, 73.1 (CH_2–Ph), 70.8 (C-1^{man}), 70.1 (C-6^{man}), 70.0 (C-7^{sia}), 69.6 (C-2^{gal}), 69.3 (C-4^{gal}), 79.8, 75.7, 74.9, 72.9, 69.2 (C-5^{gal}, C-5^{man}, C-4^{sia}, C-6^{sia}, C-8^{sia}), 64.4 (C-6^{gal}), 62.6 (C-9^{sia}), 54.0 (C-5^{sia}), 41.8 (C-3^{sia}), 22.6 ppm (CH_3^{NHAc}); MS (ESI): m/z (%): 1045.5 [M+H]$^+$ (70); HRMS (ESI): m/z calcd for [$C_{53}H_{65}N_4O_{18}$+H]$^+$: 1045.4294 [M+H]$^+$; found: 1045.4319.

*1-(3-O-(5-Acetamido-3,5-didesoxy-*D*-glycero-α-*D*-galactonon-2-ulopyranosyl))-β-*D*-galactopyranosyl-4-(α-*D*-mannopyranosyl)-1 H-1,2,3-triazole (**16**)*

Triazole **15** (155 mg, 148 μmol) was dissolved in MeOH (7 mL) and the solution was degassed by ultrasonication under an argon atmosphere. Then, Pd on charcoal (50 mg, 10 wt%) was added and, after a second degassing cycle, the argon atmosphere was replaced by hydrogen (balloon). After stirring for 1 day at RT, no conversion was observed by TLC.

$Pd(OH)_2$ on charcoal (40 mg, 20 wt%) was added and the mixture was stirred for 4 days at RT under a hydrogen atmosphere; then, the catalyst was removed by filtration through Celite and thoroughly washed with MeOH. The solvent was removed in vacuo and the crude product was purified by preparative HPLC (ACE-C_{18}-PFP) to yield the title compound (63.0 mg, 92 μmol, 62% yield) as a colorless oil. $[\alpha]_D^{24} = +5.4$ ($c = 1.25$, D_2O); ^{1}H NMR (COSY, 400 MHz, D_2O): $\delta = 8.33$ (s, 1H; H-5), 5.80 (d, $^3J_{1,2} = 9.0$ Hz, 1H; H-1^{gal}), 5.24 (d, $^3J_{1,2} = 1.9$ Hz, 1H; H-1^{man}), 4.55–4.54 (m, 1H; H-2^{man}), 4.33 (dd, $^3J_{2,3} = 9.6$ Hz, $^3J_{3,4} = 2.9$ Hz, 1H; H-3^{gal}), 4.28 (pseudo-t, $^3J_{1,2} = 9.0$ Hz, $^3J_{2,3} = 9.6$ Hz, 1H; H-2^{gal}), 4.09 (d, $^3J_{3,4} = 2.9$ Hz, 1H; H-4^{gal}), 4.00 (t, $^3J_{5,6} = 6.1$ Hz, 1H; H-5^{gal}), 3.88–3.81 (m, 5H; H-6a/b^{gal}, H-3^{man}, H-5^{sia}, H9a^{sia}), 3.78–3.73 (m, 4H; H-4^{man}, H-6a/b^{man}, H-8^{sia}), 3.70–3.66 (m, 1H; H-4^{sia}), 3.63–3.57 (m, 3H; H-5^{man}, H-6^{sia}, H-9b^{sia}), 3.44–3.41 (m, 1H; H-7^{sia}), 2.75 (dd, $^2J = 12.5$ Hz, $^3J_{3,4} = 4.7$ Hz, 1H; H-3a^{sia}), 2.01 (s, 3H; CH_3^{NHAc}), 1.83–1.79 ppm (pseudo-t, $^2J = 12.5$ Hz, $^3J_{3,4} = 12.1$ Hz, 1H; H-3b^{sia}); ^{13}C NMR (HSQC, 100.6 MHz, D_2O): $\delta = 175.0$ (C=O^{NHAc}), 173.8 (C=O^{sia}), 144.1 (C-4), 123.6 (C-5), 100.0 (C-2^{sia}), 87.7 (C-1^{gal}), 78.0 (C-5^{gal}), 75.7 (C-3^{gal}), 75.4 (C-7^{sia}), 72.8 (2C; C-1^{man}, C-5^{man}), 71.7 (C-3^{man}), 70.7 (C-4^{man}), 69.9 (C-2^{man}), 68.3 (C-4^{sia}), 68.0 (C-6^{sia}), 67.9 (C-2^{gal}), 67.6 (C-4^{gal}), 67.1 (C-8^{sia}), 62.5 (C-9^{sia}), 60.84 (C-6^{man}), 60.76 (C-6^{gal}), 51.6 (C-5^{sia}), 39.4 (C-3^{sia}), 22.0 ppm (CH_3^{NHAc}); MS (ESI): *m/z* (%): 707.3 $[M+Na]^+$ (100), 685.2 $[M+H]^+$ (51); HRMS (ESI): *m/z* calcd for $[C_{25}H_{40}N_4O_{18}+Na]^+$: 707.2235 $[M+Na]^+$; found: 707.2232; $t_r = 2.0$ min (H_2O/MeCN, 98:2→98:2 (5 min)→80:20 (15 min); flow rate = 38.00 mL min^{-1}).

*1-(β-D-Galactopyranosyl)-3-(α-D-mannopyranosyl)-indole (**20**)*

To a solution of the peracetylated diglycosylindole **19**[11a,19] (26 mg, 33 μmol) in MeOH (5 mL) was added NaOMe until the pH value reached 8.5–9.0. The mixture was stirred at RT for 18 h, neutralized with AcOH, and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel (EtOAc/MeOH, 20:1→3:1→2:1) to yield the title compound (14 mg, 32 μmol, 95% yield) as a colorless oil. $R_f = 0.45$ (1-butanol/H_2O/AcOH, 5:2:2); $[\alpha]_D^{22} = +61.0$ ($c = 1.00$, MeOH); ^{1}H NMR (COSY, HSQC, HMBC, 300 MHz, CD_3OD): $\delta = 7.84$ (dd, $^4J_{4,6} = 1.1$ Hz, $^3J_{4,5} = 7.9$ Hz, 1H; H-4^{Indol}), 7.58 (dt, $^4J_{7,5} = 0.8$ Hz, $^3J_{7,6} = 8.4$, 1H; H-7^{Indol}), 7.48 (d, $^4J_{2,1-Man} = 1.3$ Hz, 1H; H-2^{Indol}), 7.18 (ddd, $^4J_{6,4} = 1.3$ Hz, $^3J_{6,5} = 7.1$ Hz, $^3J_{6,7} = 8.4$ Hz, 1H; H-6^{Indol}), 7.08 (ddd, $^4J_{5,7} = 0.8$ Hz, $^3J_{5,6} = 7.1$ Hz, $^3J_{5,4} = 7.9$ Hz, 1H; H-5^{Indol}), 5.41 (d, $^3J_{1,2} = 9.0$ Hz, 1H; H-1^{Gal}), 5.34 (pseudo-t, $J_{app,1,2\text{-}Indol} = 1.3$ Hz, 1H; H-1^{Man}), 4.51 (dd, $^3J_{2,3} = 3.3$ Hz, $^3J_{2,1} = 2.0$ Hz, 1H; H-2^{Man}), 4.22 (pseudo-t, $J_{app} = 9.3$ Hz, 1H; H-2^{Gal}), 4.03 (dd, $J = 3.2$, 0.9 Hz, 1H; H-4^{Gal}), 3.98 (dd, $^3J_{3,2} = 3.3$ Hz, $^3J_{3,4} = 9.1$ Hz, 1H; H-3^{Man}), 3.69–3.86 (m, 7H; H-3^{Gal}, H-5^{Gal}, H-6^{Gal}, H-4^{Man}, H-6^{Man}), 3.26 ppm (ddd, $^3J_{5,6} = 2.9$ Hz, $^3J_{5,6} = 4.0$ Hz, $^3J_{5,4} = 9.4$ Hz, 1H; H-5 ^{Man}); ^{13}C NMR (DEPT, HSQC, HMBC, 75 MHz, CD_3OD): $\delta = 138.4$ (C-7a^{Indol}), 129.1 (C-3a^{Indol}), 125.0 (C-2^{Indol}), 123.3 (C-6^{Indol}), 121.5 (C-4^{Indol}), 121.2 (C-5^{Indol}), 113.4 (C-3^{Indol}), 111.6 (C-7^{Indol}), 87.2 (C-1^{Gal}), 79.2 (C-5^{Gal}), 76.3 (C-1^{Man}), 75.9, 75.8 (C-3^{Gal}, C-5^{Man}), 73.5 (C-3^{Man}), 72.1 (C-2^{Man}), 71.1 (C-2^{Gal}), 70.5 (C-4^{Gal}), 69.3 (C-4^{Man}), 62.8, 62.6 ppm (C-6^{Gal}, C-6^{Man}); MS (ESI): *m/z* (%): 464.0 $[M+Na]^+$ (100); HRMS (ESI): *m/z* calcd for $[C_{20}H_{27}NO_{10}+Na]^+$: 464.1533; found: 464.1543; *m/z* calcd for $[M+Na+H]^+$: 464.1611; found: 464.1615; IR (film): $\tilde{\nu} = 3271$, 2929, 1705, 1558, 1461, 1407, 1272, 1221, 1064, 1016, 918, 880, 794, 743, 653 cm^{-1}.

*1-[3-O-(5-Acetamido-3,5-didesoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl)-β-D-galactopyranosyl)]-3-(β-D-mannopyranosyl)-indole (**21**)*

4-Nitrophenyl-α-sialoside **9** (25 mg, 56 μmol) and diglycosylindol **20** (50 mg, 113 μmol) were dissolved in degassed Tris-HCl buffer (2 mL, 100 mM, pH 7.5). After the addition of an aqueous solution of recombinant *trans*-sialidase (400 μL, $c = 0.5$ mg mL^{-1}), the mixture was incubated at RT for 24 h. Another portion (25 mg) of compound **9** was added and the mixture was stirred for a further 48 h. The reaction was stopped by the addition of EtOH (1.5 mL). The solution was centrifuged for 30 min and the supernatant was removed and lyophilized. The crude product was purified by preparative HPLC (Luna-C18; $t_r = 20$–25 min; H_2O/MeCN, 95:5 (60 min)→80:20; flow rate = 50 mL min^{-1}) to furnish the title compound (11 mg, 15 μmol, 13% yield) as a yellowish solid. The HPLC separation also yielded unreacted acceptor **20** (22 mg, 50 μmol), thus indicating that the yield of the enzymatic reaction was 24% (brsm). $[\alpha]_D^{22} = +81.6$ ($c = 1.00$, D_2O); ^{1}H NMR (COSY, TOCSY, 600 MHz, D_2O): $\delta = 7.83$ (d, $^3J_{4,5} = 8.0$ Hz, 1H; H-4^{Indol}), 7.58 (d, $^3J_{7,6} = 8.4$ Hz, 1H; H-7^{Indol}), 7.51 (s, 1H; H-2^{Indol}), 7.29 (pseudo-t, $^3J_{6,5/7} = 7.7$ Hz, 1H; H-6^{Indol}), 7.18 (pseudo-t, $^3J_{5,4/6} = 7.6$ Hz, 1H; H-5^{Indol}), 5.62 (d, $^3J_{1,2} = 8.4$ Hz, 1H; H-1^{Gal}), 5.34 (s, 1H; H-1^{Man}), 4.55 (dd, $^3J_{2,1} = 2.3$ Hz, $^3J_{2,3} = 3.4$ Hz, 1H; H-2^{Man}), 4.27–4.32 (m, 2H; H-2^{Gal}, H-3^{Gal}), 4.08 (d, $^3J_{4,3} = 2.6$ Hz, 1H; H-4^{Gal}), 4.02 (dd, $^3J_{3,2} = 3.4$ Hz, $^3J_{3,4} = 9.2$ Hz, 1H; H-3^{Man}), 3.94–3.97 (m, 1H; H-5^{Gal}), 3.84 (ddd, $^3J_{8,9a} = 2.5$ Hz, $^3J_{8,9b} = 6.4$ Hz, $^3J_{8,7} = 9.1$ Hz, 1H; H-8^{Sia}), 3.78–3.81 (m, 2H; H-5^{Sia}, H-9a^{Sia}), 3.63–3.72 (m, 6H; H-4^{Man}, H-6^{Man}, H-4^{Sia}, H-6^{Gal}), 3.61 (dd, $^3J_{6,7} = 1.6$ Hz, $^3J_{6,5} = 10.5$ Hz, 1H; H-6^{Sia}), 3.56 (dd, $^3J_{9b,8} = 6.4$ Hz, $^3J_{9b,9a} = 12.0$ Hz, 1H; H-9b^{Sia}), 3.52 (dd, $^3J_{7,6} = 1.6$ Hz, $^3J_{7,8} = 9.1$ Hz, 1H; H-7^{Sia}), 3.23 (ddd, $^3J_{5,6} = 2.8$ Hz, $^3J_{5,6} = 5.6$ Hz, $^3J_{5,4} = 9.4$ Hz, 1H; H-5^{Man}), 2.72 (dd, $^3J_{3äqu,2} = 4.6$ Hz, $^2J_{3äqu,3ax} = 12.3$ Hz, 1H; H-3$_{äqu}^{Sia}$), 1.96 (s, 3H; $COCH_3$), 1.80 ppm (t, $^2J_{3ax,3äqu} = 12.3$ Hz, 1H; H-3$_{ax}^{Sia}$); ^{13}C NMR (HSQC, HMBC, 151 MHz, D_2O): $\delta = 174.9$ (C=O), 136.6 (C-7a^{Indol}), 127.0 (C-3a^{Indol}), 123.7 (C-2^{Indol}), 123.1 (C-6^{Indol}), 120.8 (C-5^{Indol}), 120.5 (C-4^{Indol}), 112.1 (C-3^{Indol}), 110.2 (C-7^{Indol}), 99.8 (C-1^{Gal}), 77.0 (C-5^{Gal}), 76.4 (C-3^{Gal}), 74.4 (C-1^{Man}), 74.3 (C-5^{Man}), 72.8 (C-6^{Sia}), 71.5 (C-8^{Sia}), 71.3 (C-3^{Man}), 70.0 (C-2^{Man}), 68.1 (C-7^{Sia}), 68.0, 67.7, 67.6 (C-4^{Gal}, C-4^{Man}, C-4^{Sia}), 67.4 (C-2^{Gal}), 62.5 (C-9^{Sia}), 60.8, 60.6 (C-6^{Gal}, C-6^{Man}), 51.5 (C-5^{Sia}), 39.3 (C-3^{Sia}), 21.9 ppm ($COCH_3$); MS (ESI): *m/z* (%): 755.3 $[M+Na]^+$ (100); HRMS (ESI): *m/z* calcd for $[C_{31}H_{44}N_2O_{18}+Na]^+$: 755.2487; found: 755.2514.

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