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Chemoenzymatic Synthesis of Glycoconjugates Mediated by Regioselective Enzymatic Hydrolysis of Acetylated 2-Amino Pyranose Derivatives

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Abstract: Highly regioselective deprotection of a series of 2-amino pyranose building blocks was achieved by enzymatic hydrolysis. These monodeprotected intermediates were successfully used in the synthesis of a variety of glycoconjugated derivatives with a core of glucosamine or galactosamine, including *neo*-glycoproteins and glycosphingolipids. The hydrolysis catalyzed by Acetyl xylan esterase from *Bacillus pumilus* (AXE) is suitable for the synthesis of *neo*-glycoproteins with an *N*-acetyl glucosamine core. The hydrolysis catalyzed by *Candida rugosa* lipase (CRL) was successfully applied in the preparation of new sialylated glycolipids starting from glucosamine building blocks protected as phthalimide. This chemoenzymatic approach can be used for the preparation of new glycoconjugated products with anticancer activity.

Introduction

Glycoproteins and glycolipids are involved in many physiological and pathological processes.[1] Consequently, synthetic non-natural glycoconjugates are largely investigated for developing new therapeutic strategies,[2] including the use of glycolipids with anticancer activity[3] or carbohydrate-based vaccines.[4-7]

In particular, sialylated oligosaccharides are involved in cell-cell adhesion processes[8] and many of these oligosaccharides are considered as tumor associated carbohydrate antigens (TACAs) because overexpressed in cancer cells. Specific glycosphingolipid types, which are detectable in normal cells, are more highly expressed in tumors. The high level of expression on the surface of tumour cells causes an antibody response towards these glycosphingolipids, which are therefore considered as targets for cancer therapy[9]. For example the lactosyl-ganglioside GM3 (NeuAcα3Galβ4Glcβ1Cer), is recognized as an important melanoma-associated antigen, and may have a role in metastasis.[10,11] Similarly, Sialyl-Le^X, sialyl-α-1,6-galactosamine (Sialyl-Tn antigen), and other sialylated TACAs with a core of *N*-acetyl glucosamine or galactosamine, are over-expressed in a wide variety of human cancers.[12]

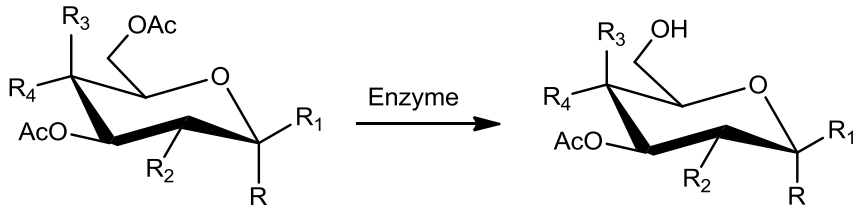
Glycoconjugated lipid analogues of GM3, obtained by sialylation of glucose at C-6, recently have

been reported as a new class of potential anticancer agents.[3] Semi-synthetic glycoproteins (*neo*-glycoproteins) can instead provide immunogens from which therapeutic agents can be derived.[13,14] In particular, *neo*-glycoproteins have been largely investigated as therapeutic anticancer vaccines[15,16] based on TACAs. Sialyl-Tn has been proposed as therapeutic vaccine against prostate cancer, and its assembly with other TACAs has been proposed for developing multivalent glycoconjugate vaccines.[17] However, the study of glycoconjugates is limited by the complex procedures required for the preparation of protected building blocks bearing free hydroxyl groups in the desired positions. In presence of glucosamine or galactosamine, the synthesis of oligosaccharides is currently performed protecting the amino as acetyl-amide,[18,19] as naturally existing in the TACAs structure. However, protection as phthalimide is often preferred.[20] At the anomeric position of the acceptor, different groups can be used, including thio-alkyl/aryl leaving groups, because after glycosylation of the free hydroxyl group, these products can be used as sugar donors for a further glycosylation reaction.[21] In addition, for the synthesis of *neo*-glycoproteins should be done the preparation of building blocks with an appropriate reactive linker at anomeric position, required for conjugation with the protein carrier. One of the most used strategies implies the use of 2-iminomethoxyethyl thioglycosides (IME) that selectively reacts with lysines on the protein surface.

The use of enzymatic regioselective hydrolysis of peracetylated sugar has been proposed as an important tool for developing simple and efficient synthesis of sugar building blocks.[22] This strategy was employed for the preparation of mannopyranose glycans with the anomeric thiocyanomethyl group, that were used for protein glycosylation after activation as anomeric IME-linker.[23,24] Peracetylated monodeprotected galactopyranoses have also been used for the preparation of sialylated glycoconjugate lipids.[25]

The most used biocatalyst for regioselective deacetylation is the lipase from *Candida rugosa* (CRL). However, other enzymes were also used. In particular, Acetyl xylan esterase from *Bacillus pumilus* (AXE), has been successfully employed in the preparation of advanced building blocks by

regioselective deacetylation of acetylated disaccharides.[26] The use of this chemoenzymatic approach has never been investigated for the preparation of glyco-derivatives (*neo*-glycoproteins and glycolipids) using TACAs with a core of glucosamine or galactosamine, such as the STn antigen. Consequently, in this work, we have studied the regioselective enzymatic hydrolysis of 2-amino-pyranoside derivatives (Scheme 1), exploring the effect on the enzyme activity and selectivity induced by the presence of different reactive groups in anomeric position and by the protection of the 2-amino function.



1-11 **12-21**

Scheme 1. Regioselective hydrolysis of acetylated glucosamine and galactosamine derivatives **1-11** catalyzed by immobilized CRL and AXE (Table 1).

The results obtained demonstrated that this approach is versatile and can be conveniently used for the preparation of different building blocks bearing only a free hydroxyl group at C-6 with presenting different functional groups at anomeric position, including the thio-cyanomethyl group currently employed for protein glycosylation. These intermediates can be conveniently glycosylated with different sugar donors, allowing the preparation of glycans used for the synthesis of glycoconjugated derivatives (*neo*-glycoproteins and glycosphingolipids) including sialylated products such as the STn antigen.

Results and Discussion

Enzymatic hydrolysis of different substrates

In this work, two immobilized enzymes have been compared (CRL and AXE) for the preparation of various glucosamine and galactosamine building blocks. A screening has been performed at low substrate concentration (10 mM) and in presence of 20% of acetonitrile in order to ensure the complete solubility and hydrolysis of the substrates (Table 1).

Table 1. Enzymatic Hydrolysis of Different Derivatives of Glucosamine and Galactosamine

Substrates	Products	CRL: yield (h)	AXE: yield (h)
1: R,R_4=OAc; R_1,R_3=H; R_2=NHAc	**12**	94% (24)	10% (3)[a]
1[b]: R,R_4=OAc; R_1,R_3=H; R_2=NHAc	**12**	80% (24)	-
2: R,R_3=H; R_1=OMe; R_2=NHAc R_4=OAc	**13**	70% (24)	94% (24)
3: R,R_3=H; R_1=SCH_2CN; R_2=NHAc; R_4=OAc	**14**	68% (48)	90% (24)
4: R,R_3=H; R_1=SEt; R_2=NHAc; R_4=OAc	**15**	<10% (48)[a]	85% (12)
5: R,R_3=H; R_1=SPh; R_2=NHAc; R_4=OAc	**16**	<10% (48)[a]	65% (6)
6: R,R_4=OAc; R_1,R_3=H; R_2=NPhth	**17**	76% (6)	<10% (24)[a]
7: R,R_3=H; R_1=OMe; R_2=NPhth; R_4=OAc	**18**	85% (4)	12% (8)[a]
8: R,R_3=H; R_1=SEt; R_2=NPhth; R_4=OAc	**19**	64% (24)	30% (96)[a]
9: R,R_3=H; R_1=SPh; R_2=NPhth; R_4=OAc	**20**	38% (24)	<10% (194)[a]
10[c]: R,R_3=OAc; R_1,R_4=H; R_2=NHAc	**21**	70% (30)	-
11: R,R_4=H; R_1=SEt; R_2=NPhth; R_3=OAc	**22**	80% (5)	-

[a]Yield evaluated by HPLC analysis without isolation of the product.
[b]α/β=5/1. [c]α/β=85/15.
Experimental conditions: substrate 10 mM, volume 15 mL, phosphate buffer 50 mM, acetonitrile 20% (for substrates **6** and **7**, 25% of acetonitrile was used), pH 5, r.t., enzyme 1 g of CRL-OD (1200 UI/g) or AXE (90 UI/g).

The esterase AXE was used immobilized by covalent attachment on epoxy carrier since covalent immobilization generally ensures the stabilization of enzymes such as esterases or proteases. In the case of CRL, this enzyme was used immobilized by adsorption on hydrophobic carrier because this method is appropriate to obtain good stability and activity of lipases.[22]

Except for 2-deoxy-2-acetamido-1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside **1** (since AXE was reported to catalyze hydrolysis the anomeric position of pyranoses with acetoxy group),[27] considering the other *N*-acetylated substrates **2-5**, the esterase AXE allowed best performances. In fact, the pure α-anomer of **1**, with the anomeric acetyl leaving group, can be used as substrate for immobilized CRL according to the procedure previously reported.[28,29] In this work, we used CRL immobilized on Sepabeads-OD (a C18 hydrophobic carrier) and the obtained results (Table 1) were similar (94% of 2-deoxy-2-acetamido-1,3,4-tri-*O*-acetyl-α-D-glucopyranose **12**) to those previously reported using CRL immobilized on octyl-agarose (a C8 hydrophobic carrier). However, CRL-Sepabeads-OD permitted much higher reaction rate providing almost quantitative hydrolysis of **1** in 24 hours. When the same reaction condition was used, the CRL immobilized on C8 agarose provided only 75% of the substrate hydrolyzed in 48 hours. The enzymatic hydrolysis of **1** was performed up to 20 mM concentration (about 8 g/L) and 92% yield of **12** was obtained after 24 hours. In addition, the hydrolysis of **1** with CRL was also tested using the α and β mixture (5/1) avoiding the separation of the two anomers after the chemical synthesis of this substrate (Table 1). The enzyme can be completely selective to provide the pure α anomer of **12** in 80% yield, corresponding to almost complete hydrolysis of the α-form of **1**. In this case, the β anomer remains completely unreacted and can be easily separated from the α-**12**.

Using AXE, methyl 2-deoxy-2-acetamido-3,4-di-*O*-acetyl-β-D-glucopyranoside **13** was prepared in 94% yield (Table 1), much better compared with the result obtained by means of CRL (70% yield). Similarly, yields ranging from 65% to 90% of various sugar building blocks have been obtained with AXE, starting from *N*-acetyl glucosamine derivatives with an anomeric *S*-alkyl group, including the thio-cyanomethyl reactive linker used for the glycosylation of proteins. In fact, cyanomethyl 2-deoxy-2-acetamido-3,4,-di-*O*-acetyl-1-thio-β-D-glucopyranoside **14** was prepared in 90% yield. This reaction was scaled up to 50 mM concentration (about 20 g/L) of substrate **3** (see Table S1 in Supporting Information).

Similar results have been obtained in the hydrolysis of ethyl 2-deoxy-2-acetamido-3,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside **4** providing ethyl 2-deoxy-2-acetamido-3,4-di-*O*-acetyl-1-thio-β-D-glucopyranoside **15** (85% yields). Lower yield (Table 1) was obtained in the hydrolysis of phenyl 2-deoxy-2-acetamido-3,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside **5** (65% yield of product **16**).

Also in the hydrolysis of the 2-deoxy-2-phthalimido glucopyranoside derivatives **6-9**, good or very good yields were obtained, but in these cases CRL provided the best results. Starting from the 2-deoxy-2-phthalimido-1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose **6**, 76% yield of 2-deoxy-2-phthalimido-1,3,4-tri-*O*-acetyl-α-D-glucopyranose **17** (with the anomeric *α*-*O*-acetyl group) was obtained in 6 h of reaction (Table 1). As observed for other substrates, also in this case the reaction catalyzed by the Octyl-agarose derivative of CRL proceeded much slower (18 hours for complete hydrolysis of **6**) compared with the CRL-OD derivative. This reaction was performed by increasing the substrate concentration up to 10 g/L (see Table S2 Supporting Information).

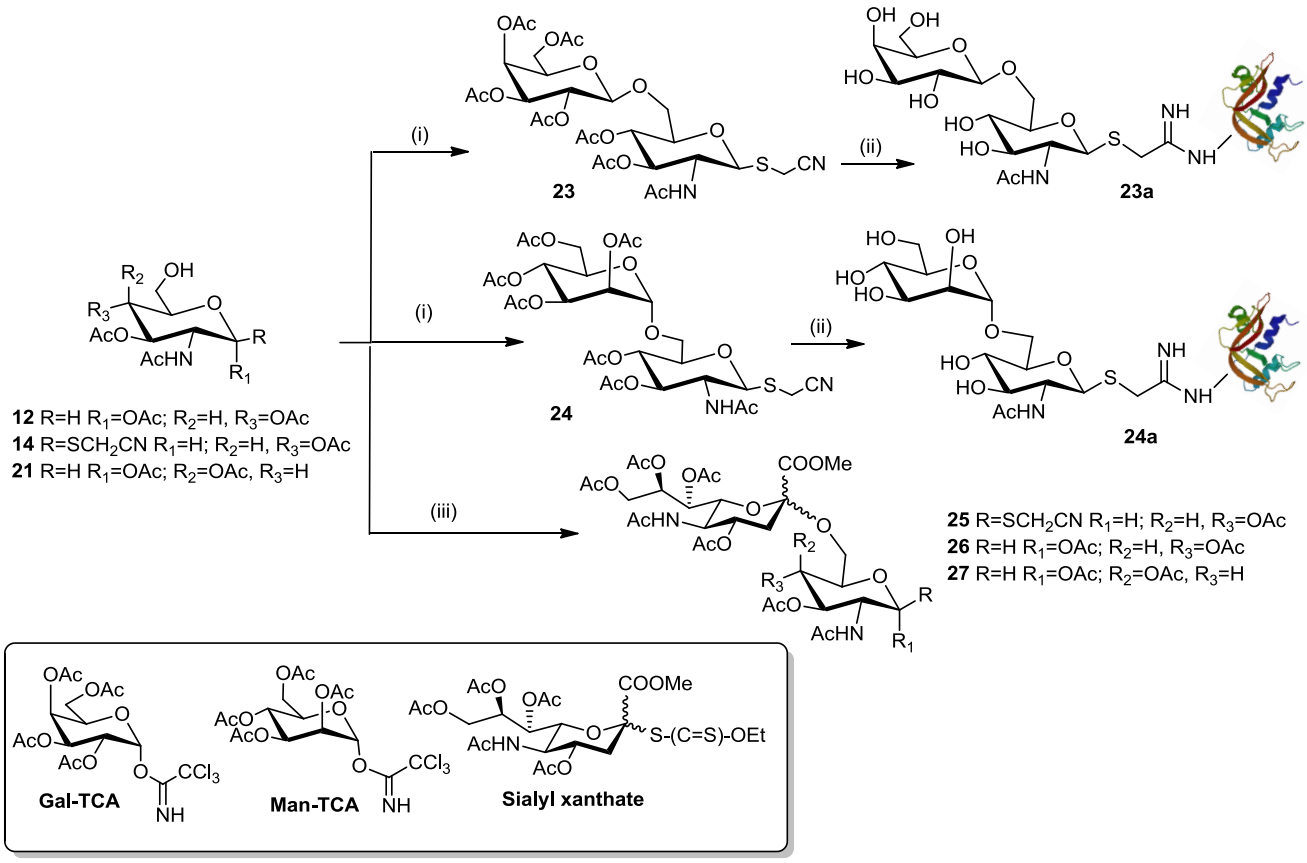
Products with one free hydroxyl group at C-6 in good yields have also been obtained in the hydrolysis of methyl 2-deoxy-2-phthalimido-3,4,6-tri-*O*-acetyl-β-D-glucopyranoside **7**, (85% of product **18**), and ethyl 2-deoxy-2-phthalimido-3,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside **8**, (64% of product **19**). About 90% of these two substrates, characterized by a little O- or S-alkyl anomeric group, were hydrolyzed in 4 and 24 hours, respectively. Lower yield was obtained in the hydrolysis of phenyl 2-deoxy-2-phthalimido-3,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside **9**, because this reaction proceeded slowly (38% yield of product **20** in 24 hours).

Finally, the enzymatic hydrolysis of galactosamine derivatives was also investigated. The hydrolysis of 2-deoxy-2-acetamido-1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose **10** (Table 1), obtained by chemical synthesis in α and β mixture (85/15), has been performed using CRL. This reaction provided 2-deoxy-2-acetamido-1,3,4-tri-*O*-acetyl-α-D-galactopyranose **21** (with the anomeric *O*-acetyl group) with 70% yield in pure α form (the β anomer is not hydrolyzed by CRL) and was scaled up by improving the substrate concentration up to 10 g/L (70% yield after purification). The hydrolysis of the ethyl 2-deoxy-2-phthalimido-3,4,6-tri-*O*-acetyl-1-thio-β-D-galactopyranoside **11** proceeded

much faster compared with the fully acetylated galactosamine **10**, and ethyl 2-deoxy-2-phthalimido-3,4-di-*O*-acetyl-1-thio-β-D-galactopyranoside **22** was obtained in very good yield (80% after purification) after only 5 hours of reaction (Table 1).

Synthesis of disaccharides and glycoconjugated derivatives

The different building blocks prepared by enzymatic hydrolysis have been then used as intermediates for the preparation of disaccharides. Starting from **14**, products **23** and **24** (with the anomeric thio-cyanomethyl reactive linker) have been synthesized in almost pure anomeric form with good yields (Scheme 2).



Scheme 2. (i) **a) 14**, **Gal-TCA**, $BF_3{\cdot}Et_2O$, CH_2Cl_2, N_2, 0 °C, (**23**, 68% yield; β>95%); **b) 14**, **Man-TCA**, $BF_3{\cdot}Et_2O$, CH_2Cl_2, N_2, 0°C, (**24**, 63% yield; α>95%); (ii) **a)** MeONa/MeOH, r.t., 24 h, 50% yield IME-glycans; **b)** sodium tetraborate buffer, pH 9.5, 25 °C, molar ratio IME-glycan/ribonuclease A 100:1, 6 h; **23a** yield 81%; **24a** yield>98%; (iii) **a) 14**, sialyl xanthate, NIS, TfOH, CH_3CN; -40 °C; **25** yield 55% (α/β 3/2); **b) 12**, sialyl xanthate, PhSCl, AgOTf, CH_3CN and CH_2Cl_2 (2/1), N_2; -68 °C; **26** yield 60% (α/β 2/1); **c) 21**, sialyl xanthate, PhSCl, AgOTf, CH_3CN and CH_2Cl_2 (2/1), N_2; -68 °C; **27** yield 64% (α/β 1/1).

These compounds, after activation to obtain the corresponding deprotected iminomethoxyethyl thioglycosides, (see Supporting Information), have been used for the preparation of *neo*-glycoproteins **23a** and **24a** in 80% and 100% yields respectively, as resulting by MS analysis (Figure 1) considering the % of glycosylated protein (RNase-A selected as model of carrier protein). These results demonstrate that the proposed approach, mediated by regioselective hydrolysis catalyzed by AXE, is suitable for the preparation of *neo*-glycoproteins with glycans having a glucosamine core.

However, when the building block **14** was used for the preparation of sialylated glyco-derivatives (potentially advantageous for the preparation of sialylated *neo*-glycoprotein), the glycosylation with sialyl xanthate afforded product **25** (55% yield) in 3/2 α/β mixture (Scheme 2). Similarly, sialylation of *N*-acetyl glucosamine and galactosamine building blocks **12** and **21**, allowed preparation of the disaccharides **26** and **27** as a mixture of α/β anomers.

The difficulties intrinsic in the stereo-controlled synthesis of α-sialosides have been largely reported and complex procedures are required in order to obtain pure or almost pure anomers.[30] In fact, α/β mixtures are obtained in different ratio using monodeprotected sugar acceptors, regardless the protection used for the C-2 amino group of the pyranose acceptor or using precursors with the 2-azido group.[31,32] In our work, this effect was observed regardless the kind of sialyl-donor used in the glycosylation of 2-deoxy-2-acetamido glucose or galactose building blocks. In addition, the sialylation of **21** was investigated in different conditions (including the use of sialyl chloride donor), but in all cases similar yields and an anomeric mixtures of **27** was obtained (see Supporting Information).

Surprisingly, high stereoselectivity was obtained when *N*-phthalimido glucosamine or galactosamine intermediates were considered for the sialylation after enzymatic deprotection at C-6. Accordingly, it was possible to perform the preparation of sialyl-disaccharides with different reactive groups at C-1, in pure or almost pure α-form (Scheme 3). Product **28** (with 1-α-acetoxy) was obtained in 70% yield as almost pure α anomer, allowing results much better compared with those previously reported.[31] Similarly, pure α anomer of **29** (with 1-β-methoxy) was obtained in 64% yield.

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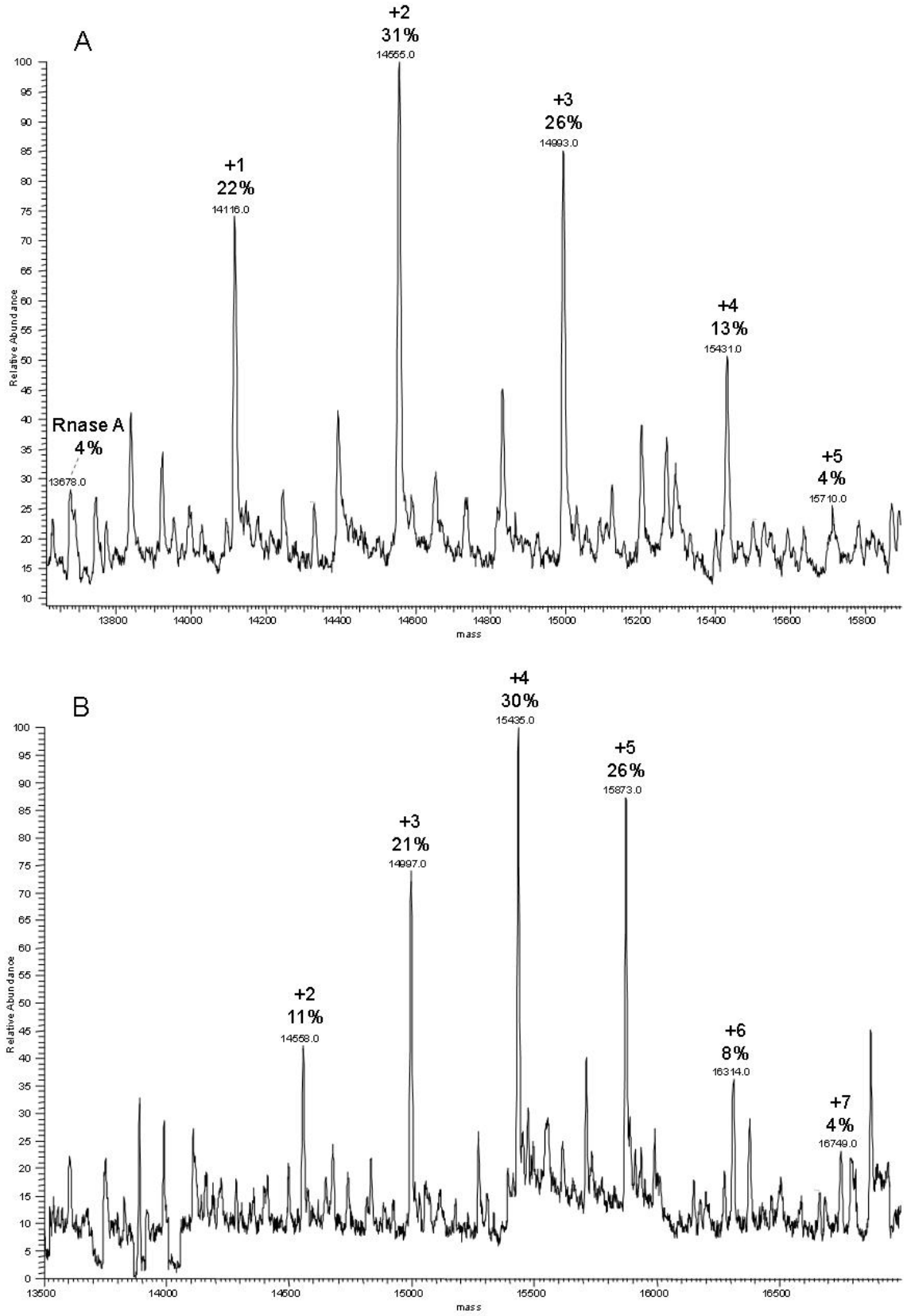
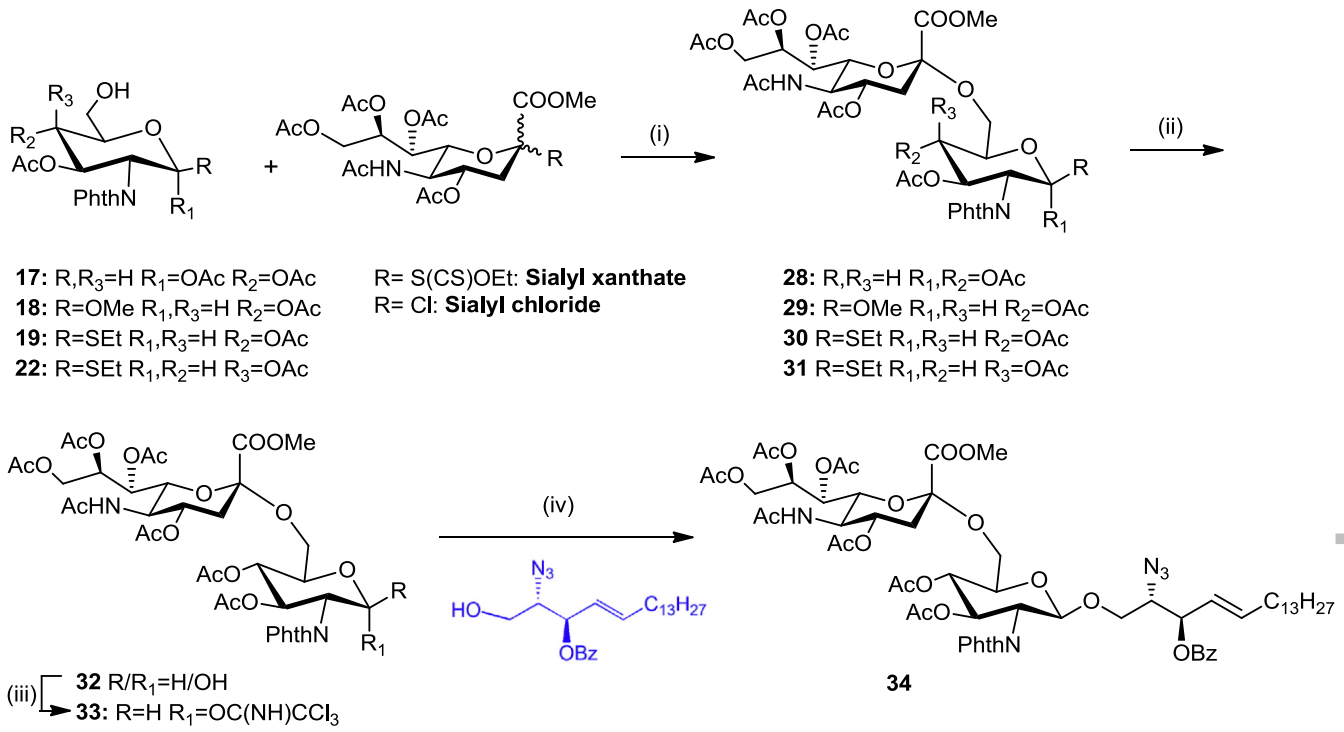


Figure 1. MS spectra obtained in the glycosylation of RNase A with the glycans **IME-23** and **IME-24** to obtain *neo*-glycoproteins **23a** (A) (average number of incorporated saccharides: 2.3) and **24a** (B) (average number of incorporated saccharides: 4.1).



Scheme 3. (i) **a)** sialyl xanthate, PhSCl, AgOTf, CH_3CN and CH_2Cl_2 (2/1), N_2, -68°C; **28** yield 70% (>98%); **29** yield 64% (>98%); **30** yield 32% (>98%); **31** yield 30% (>98%); **b)** sialyl chloride, AgOTf, CH_2Cl_2, N_2, -40 °C; **30** yield 65% (95%); **31** yield 70% (95%). (ii) **28**, $NH_2CH_2CH_2NH_2$, AcOH, THF, r.t., 3 h, **32** (75% crude). (iii) **32**, CCl_3CN, DBU, CH_2Cl_2, -5 °C, 2 h, **33** (60%, α/β 4/1). (iv) **33**, 3-*O*-benzoyl-azidosphingosine, $BF_3{\cdot}Et_2O$, CH_2Cl_2, -30 °C, 1.5 h, **34** (61%).

Much poorer performances were observed when sialyl xanthate was used as donor for sialylation of *N*-phthalimido- glucosamine **19** and galactosamine **22** (with 1-β-thioethyl)**,** probably as consequence of the activation of the anomeric S-alkyl group in the reaction condition used that causes the formation of by-products. In fact, starting from *N*-phthalyl-glucosamine **19**, pure **30** was obtained only in 32% yield, and the sialyl-Tn derivative **31** was produced in 30% yield from *N*-phthalyl-galactosamine **22** (see Supporting Information). For improving the yield in the sialylation of *N*-phthalimido building blocks, the sialyl chloride was investigated as donor in the glycosylation of intermediates **19** and **22** (Scheme 3). In this condition, 65% and 70% yields were obtained respectively for product **30** and sialyl-Tn precursor **31** (in both cases as 95/5 mixture of α/β isomers).

These results suggest that the presence of an acetamido group at position C-2, unlike the 2-phthalimido group, could be responsible for the formation of the β-anomer during sialylation. In particular, the 2-acetamido group could be involved in the formation of a hydrogen bond with the carboxy-ester of the sialic acid donor. Accordingly, this effect was not observed in the glycosylation of *N*-acetamido-glucosamine intermediate **14** with the **Gal-TCA** or **Man-TCA**. In fact, disaccharides **23** and **24** were obtained in pure β and α-form, respectively (Scheme 2). Consequently, the use of 2-phthalimido pyranoses should be preferred for the synthesis of sialyl derivatives.

Finally, starting from the sialylated disaccharide **28** (Scheme 3), after removing the anomeric acetyl group (yielding 75% of the crude product **32**) and activation as trichloroacetimidate (yield in 60% of **33** after purification), the glycosylation of the 3-*O*-benzoyl-azidosphingosine building block provided the sialylated glycosphingolipid **34** in 61% yield.

Conclusions

In summary, a new chemoenzymatic synthetic strategy has been investigated and optimized for the preparation of *neo*-glycoproteins and glycolipids with a core of glucosamine or galactosamine, which are interesting as potential anticancer agents. A regioselective enzymatic hydrolysis can be conveniently used for the synthesis of 2-amino-pyranose building blocks bearing free hydroxyl group in primary position, starting from different acetylated 2-deoxy-2-acetamido or 2-deoxy-2-phthalimido gluco- and galactopyranoses. However, depending on the product desired, the appropriate starting material (substrate) and enzyme should be selected.

Thus, AXE can be used for the synthesis of *neo*-glycoproteins with an *N*-acetyl glucosamine core. In fact, the hydrolysis catalyzed by this enzyme provided different intermediates with a β-1-thio-(*S*-alkyl) group in very good yields (from 65% to 95%). For example, cyanomethyl 2-deoxy-2-acetamido-3,4,-di-*O*-acetyl-1-thio-β-D-glucopyranoside **14** was prepared in 90% yield and, after glycosylation, two disaccharides with the thiocyanomethyl reactive linker at anomeric position were obtained and used for the preparation of *neo*-glycoproteins.

CRL provides C-6 monodeprotected 2-*N*-phthalyl pyranose building blocks in good yields (from 65% to 85%) with different groups at anomeric position that can be used for the preparation of new sialylated glycoderivatives. Accordingly, the synthesis of protected Sialyl-Tn antigen **31** has been performed in two steps (56% yield) while the glycosphingolipid **34** has been prepared in 3 steps in 40% yield.

The monodeprotected compounds obtained in this work can be used as intermediates for the preparation of different products as potential new anticancer agents. Different glycosphingolipids will be prepared using this synthetic route and investigated as anticancer GM3 analogues.

Experimental Section

Materials and general experimental methods

2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranoside (**1**) and 2-acetamido-2-deoxy-D-galactopyranoside, *Candida rugosa* lipase were purchased from Sigma-Aldrich (Italy, Milan). The octadecyl-Sepabeads^{TM} was kindly supplied by Resindion (Italy). Immobilized AXE was kindly donated by ACS Dobfar (Italy). All chemicals were purchased as reagent grade and used without further purification. All chemical reactions were carried out under N_2 atmosphere and anhydrous conditions with freshly distilled solvents, unless otherwise noted. Reactions were monitored by thin-layer chromatography (TLC) on a pre-coated plate of silica gel 60 F254 (Merck) and detection by staining with sulfuric acid. Solvents were evaporated under reduced pressure and below 40 °C (water bath). Column chromatography was performed on silica gel 60 (230-400 mesh, Merck). ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz with Bruker AVANCE DRX 400 spectrometer. The chemical shifts were referenced to the solvent peak, 7.26 ppm (^{1}H) and 77.16 ppm (^{13}C) for $CDCl_3$ at 25 °C, and coupling constants were given in Hz. High-resolution mass spectra (HRMS) were recorded with a Bruker micro-TOF spectrometer in electrospray ionization (ESI) mode, using Tuning-Mix as reference. All the new compounds were fully characterized by ^{1}H and ^{13}C NMR, as well as HRMS. All the known products were characterized only by ^{1}H NMR by comparison with literatures.

Synthesis of substrates 1-11

Synthesis of substrates **1-11** has been performed using conventional chemical procedures. Experimental detail and analytical characterization are reported in the Supporting Information.

Immobilization of Candida rugosa lipase (CRL)

Immobilization of CRL was performed slightly modifying the procedure previously reported.[33] Briefly, the crude extract of CRL (2 g) was diluted in 25 mM phosphate buffer pH 7 (32 mL) and kept stirring for 30 min. 5 g of octadecyl-Sepabeads™, previously washed with the same buffer, was added and the suspension was stirred at r.t. for 3 h. The enzyme derivative (CRL-OD) was then filtered and washed with distilled water.

The activity of the enzyme was measured at pH 7.0 and r.t. in the hydrolysis of tripropionin according to the literature.[34]

Enzymatic hydrolysis

The enzymatic hydrolysis of substrates **1-11** (10 mM) was carried out in 50 mM phosphate buffer (15 mL) containing 20% acetonitrile (for **6** and **7**, 25% of acetonitrile for complete solubility of the substrates) under mechanical stirring. The reaction started after the addition of 1 g of immobilized enzyme CRL-OD (1200 UI/g) or AXE (90 UI/g). During the reaction, the pH of the solution was maintained by automatic titration. The course of the hydrolysis reaction was monitored by HPLC (HPLC analysis: 30-50% acetonitrile in phosphate buffer (10 mM) at pH 4, flow rate 1.0 mL/min, λ = 210 nm) and TLC. After complete or almost-complete consumption of the substrate, the reaction was stopped by biocatalyst filtration, and the obtained products were isolated by extraction with ethyl acetate followed by purification by flash chromatography (product **12**, **13** and **14**: CH_2Cl_2-MeOH, 95:5, product **15**: CH_2Cl_2-Et_2O, 7:3, product **16**: EtOAc-Hexane, 4:1, product **17**: Toluene-EtOAc,

3:2, product **18** and **21**: Hexane-EtOAc, 1:1, product **19**, **20** and **22**: Hexane-EtOAc, 3:2) and characterized by NMR spectra and mass spectrometry.

Analytical data of enzymatic products 12-22 (^{1}H, ^{13}C NMR and MS)

2-*acetamido-1,3,4-tri-O-acetyl-2-deoxy-α-D-glucopyranoside (**12**).* R_f = 0.36 (CH_2Cl_2-MeOH, 95:5). The ^{1}H NMR is accordance with the literature previously reported.[28] ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.30 (d, *J*=3.3 Hz, 1H, H-1), 5.64 (d, *J*=8.2 Hz, 1H, NH), 5.32 (t, *J*=9.8 Hz, 1H, H-3), 5.18 (t, *J*=9.6 Hz, 1H, H-4), 4.49 (m, 1H, H-2), 3.84 (m, 1H, H-5), 3.60, 3.74 (dd, 2H, H-6), 2.22 (s, 3H, OAc), 2.11 (s, 3H, OAc), 2.05 (s, 3H, OAc), 1.96 (s, 3H, NAc).

*Methyl 2-acetamido-3,4-di-O-acetyl-2-deoxy-β-D-glucopyranoside (**13**).* The ^{1}H NMR is in accordance with the literature previously reported.[35] R_f = 0.30 (CH_2Cl_2-MeOH, 95:5). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.90 (d, *J*=8.4 Hz, 1H, NH), 5.30 (dd, *J*=10.2, 9.8 Hz, 1H, H-3), 5.10 (t, *J*=9.8 Hz, 1H, H-4), 4.60 (d, *J*=8.2 Hz, 1H, H-1), 4.22 (dd, *J*=12.6, 4.0 Hz, 1H, H-6a), 4.16 (dd, *J*=12.4, 2.4 Hz, 1H, H-6b), 3.82 (dd, *J*=10.3, 8.4 Hz, 1H, H-2), 3.70-3.78 (m, 1H, H-5), 3.50 (s, 3H, OCH_3), 2.08 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.98 (s, 3H, NAc).

*Cyanomethyl 2-acetamido-3,4-di-O-acetyl-2-deoxy-1-thio-β-D-glucopyranoside (**14**).* R_f = 0.28 (CH_2Cl_2-MeOH, 95:5). The ^{1}H NMR is accordance with the literature previously reported.[36] ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.80 (d, *J*=9.5 Hz, 1H, NH), 5.20 (t, *J*=9.5 Hz, 1H, H-3), 5.13 (t, *J*=9.5 Hz, 1H, H-4), 4.74 (d, *J*=10.3 Hz, 1H, H-1), 4.20 (td, *J*=10.3, 9.5 Hz, 1H, H-2), 3.74 (d, *J*=17.3 Hz, 1H, CH_2CN), 3.80-3.71 (m, 1H, H-6a), 3.66-3.61 (m, 1H, H-5), 3.60-3.58 (m, 1H, H-6b), 3.32 (d, *J*=17.3 Hz, 1H, CH_2CN), 2.07 (s, 3H, OAc), 2.06 (s, 3H, OAc), 1.97 (s, 3H, NAc).

*Ethyl 2-acetamido-3,4-di-O-acetyl-2-deoxy-1-thio-β-D-glucopyranoside (**15**).* The product is as a white solid, m.p.: 65-68 °C. R_f = 0.36 (CH_2Cl_2-Et_2O, 7:3). ^{1}H NMR (400 Hz, $CDCl_3$): δ 5.85 (d, *J*=11.4

Hz, 1H, NH), 5.20 (t, *J*=8.5 Hz, 1H, H-3), 5.03 (t, *J*=8.5 Hz, 1H, H-4), 4.61 (d, *J*=10.4 Hz, 1H, H-1), 4.10 (dd, *J*=19.3, 10.4 Hz, 1H, H-2), 3.76-3.52 (m, 3H, H-5, H-6), 2.76-273 (m, 2H, SCH_2CH_3), 2.04 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.95 (s, 3H, NAc), 1.25 (t, *J*=7.4 Hz, 3H, SCH_2CH_3). ^{13}C NMR (100 Hz, $CDCl_3$): δ 14.87 (SCH_2CH_3), 20.78, 20.84 (OAc), 23.38 (NHAc), 24.12 (SCH_2CH_3), 53.33 (C-2), 61.74 (C-6), 68.92 (C-4), 73.95 (C-3), 78.44 (C-5), 84.43 (C-1), 170.12, 170.29, 171.27 (C=O). ESI-HRMS (m/z) calcd for $C_{14}H_{23}NNaO_7S$ $[M+Na]^+$: 372.1093, found: 372.1078.

*Phenyl 2-acetamido-3,4-di-O-acetyl-2-deoxy-1-thio-β-D-glucopyranoside (**16**).* The product is as a white solid, m.p.: 88-91 ℃. R_f = 0.30 (EtOAc-Hexane, 4:1). ^1H NMR (400 Hz, $CDCl_3$): δ 7.48-7.46 (m, 2H, ArH), 7.33-7.30 (m, 3H, ArH), 5.58 (d, *J*=9.4 Hz, 1H, NH), 5.24 (t, *J*=8.8 Hz, 1H, H-3), 5.01 (t, *J*=9.8 Hz, 1H, H-4), 4.88 (d, *J*=10.2 Hz, 1H, H-1), 4.04 (dd, *J*=20.3, 10.4 Hz, 1H, H-2), 3.74-3.69 (m, 1H, H-5), 3.62-3.52 (m, 2H, H-6), 2.04 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.98 (s, 3H, NAc). ^{13}C NMR (100 Hz, $CDCl_3$): δ 20.78, 20.84 (OAc), 23.49 (NHAc), 53.70 (C-2), 61.88 (C-6), 68.78, 73.76, 78.47 (C-3, C-4, C-5), 86.70 (C-1), 129.24, 132.50 (aromatic C), 170.06, 170.11, 171.23 (C=O). ESI-HRMS (m/z) calcd for $C_{18}H_{23}NNaO_7S$ $[M+Na]^+$: 420.1093, found: 420.1069.

*1,3,4-tri-O-acetyl-2-phthalimido-2-deoxy-α-D-glucopyranoside (**17**).* R_f = 0.36 (Toluene-EtOAc, 3:2). The ^1H NMR is accordance with the literature previously reported.[37] ^1H NMR (400 Hz, $CDCl_3$): δ 7.87-7.83 (m, 2H, phthalimido protons), 7.75-7.72 (m, 2H, phthalimido protons), 6.60 (dd, *J*=11.5, 9.1 Hz, 1H, H-3), 6.29 (d, *J*=3.3 Hz, 1H, H-1), 5.15 (t, *J*=9.6 Hz, 1H, H-4), 4.71 (dd, *J*=11.6, 3.3 Hz, 1H, H-2), 4.15-4.08 (m, 1H, H-5), 3.82-3.62 (2m, 2H, H-6), 2.09 (s, 3H, OAc), 2.08 (s, 3H, OAc), 1.88 (s, 3H, OAc).

*Methyl 3,4-di-O-acetyl-2-phthalimido-2-deoxy-β-D-glucopyranoside (**18**).* The product is as colorless oil. R_f = 0.40 (Hexane-EtOAc, 1:1). ^1H NMR (400 Hz, $CDCl_3$): δ 7.88-7.84 (m, 2H, phthalimido protons H), 7.75-7.73 (m, 2H, phthalimido protons), 5.83 (dd, *J*=9.8, 1.6 Hz, 1H, H-3),

5.34 (d, *J*=9.6 Hz, 1H, H-1), 5.12 (t, *J*=9.2 Hz, 1H, H-4), 4.28 (dd, *J*=8.8, 2.6 Hz, 1H, H-2), 3.84-3.81 (m, 1H, H-5), 3.71-3.64 (m, 2H, H-6), 3.45 (s, 3H, OCH_3), 2.07 (s, 3H, OAc), 1.87 (s, 3H, OAc). ^{13}C NMR (100 Hz, $CDCl_3$): δ 20.67 (OAc), 20.92 (OAc), 50.65 (C-2), 60.52 (OMe), 61.21 (C-6), 66.45 (C-4), 70.17 (C-3), 72.80 (C-5), 90.63 (C-1), 124.07, 134.99 (aromatic C), 167.60-171.29 (C=O). ESI-HRMS (m/z) calcd for $C_{19}H_{21}NNaO_9$ $[M+Na]^+$: 430.1114, found: 430.1126.

*Ethyl 3,4-di-O-acetyl-2-phthalimido-2-deoxy-1-thio-β-D-glucopyranoside (**19**).* R_f = 0.41 (Hexane-EtOAc, 3:2). The ^1H NMR is accordance with the literature previously reported.[38] ^1H NMR (400 MHz, $CDCl_3$): δ 7.75-7.95 (m, 4H, phthalimido protons), 5.90 (t, *J*=8.8 Hz, 1H, H-3), 5.52 (d, *J*=10.5 Hz, 1H, H-1), 5.16 (t, *J*=8.6 Hz, 1H, H-4), 4.40 (t, *J*=9.5 Hz, 1H, H-2), 4.31 (dd, *J*=11.7, 5.1 Hz, 1H , H-5), 3.72 (m, 1H, H-6a), 3.68 (m, 1H, H-6b), 2.60-2.82 (m, 2H, SCH_2CH_3), 2.15 (s, 3H, OAc), 1.89 (s, 3H, OAc), 1.22 (t, *J*=7.4 Hz, 3H, SCH_2CH_3).

*Phenyl 3,4-di-O-acetyl-2-phthalimido-2-deoxy-1-thio-β-D-glucopyranoside (**20**).* R_f = 0.38 (Hexane-EtOAc, 3:2). The ^1H NMR is accordance with the literature previously reported.[38] ^1H NMR (400 MHz, $CDCl_3$): δ 7.94-7.75 (m, 4H, phthalimido protons), 7.48-7.26 (m, 5H, Ph), 5.85 (dd, *J*=9.5 Hz, 1H, H-3), 5.79 (d, *J*=10.6 Hz, 1H, H-1), 5.14 (t, *J*=9.6 Hz, 1H, H-4), 4.39 (t, *J*=10.6 Hz, 1H, H-2), 3.84–3.61 (m, 3H, H-5, H-6), 2.05 (s, 3H, OAc), 1.87 (s, 3H, OAc).

*2-acetamido-1,3,4-tri-O-acetyl-2-deoxy-α-D-galactopyranoside (**21**).* R_f = 0.30 (EtOAc-Hexane, 1:1). The ^1H NMR is accordance with the literature previously reported.[28] ^1H NMR (400 MHz, CDCl3): δ 6.25 (d, *J*=3.6 Hz, 1H, H-1), 5.58 (bd, 1H, NH), 5.45 (bdd, 1H, H-4), 5.30 (dd, *J*=8.7, 3.1 Hz, 1H, H-3), 4.80 (ddd, *J*=11.0, 9.8, 3.6 Hz, 1H, H-2), 4.08 (t, *J*=12.8 Hz, 1H, H-5), 3.50-3.64 (m, 2H, H-6), 2.23 (s, 3H, OAc), 2.18 (s, 3H, OAc), 2.08 (s, 3H, OAc), 1.96 (s, 3H, NAc).

*Ethyl 3,4-di-O-acetyl-2-phthalimido-2-deoxy-1-thio-β-D-galactopyranoside (**22**).* The product is as a white solid, m.p.: 78-80 °C. R_f = 0.35 (Hexane-EtOAc, 3:2). ^{1}H NMR (400 Hz, $CDCl_3$): δ 7.87-7.85 (m, 2H, phthalimido protons), 7.76-7.74 (m, 2H, phthalimido protons), 5.85 (dd, *J*=10.6, 3.2 Hz, 1H, H-3), 5.50 (d, *J*=3.0 Hz, 1H, H-4), 5.48 (d, *J*=10.4 Hz, 1H, H-1), 4.63 (t, *J*=10.5 Hz, 1H, H-2), 3.98-3.95 (m, 1H, H-5), 3.78, 3.55 (2m, 2H, H-6), 2.75-2.67 (m, 2H, SCH_2CH_3), 2.22 (s, 3H, OAc), 1.86 (s, 3H, OAc), 1.21 (t, *J*=7.2 Hz, 3H, SCH_2CH_3). ^{13}C NMR (100 Hz, $CDCl_3$): δ 15.11 (SCH_2CH_3), 20.67 (OAc), 20.92 (OAc), 24.71 (SCH_2CH_3), 50.65 (C-2), 61.21 (C-6), 67.97 (C-4), 69.07 (C-3), 77.69 (C-5), 81.85 (C-1), 123.77, 123.86, 134.42, 134.47, 134.54 (aromatic C), 167.60, 168.11, 169.80, 171.29 (C=O). ESI-HRMS (m/z) calcd for $C_{20}H_{23}NNaO_8S$ $[M+Na]^+$: 460.1042, found: 460.1025.

Synthesis of disaccharides 23-31

*Cyanomethyl (2',3',4',6'-tetra-O-acetyl-β-D-galactopyranosyl)-(1→6)-2-acetamido 3,4-di-O-acetyl-2-deoxy-1-thio-β-D-glucopyranoside (**23**).* 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranose trichloroacetimidate (0.137 g, 0.278 mmol) and compound **14** (0.05 g, 0.139 mmol) were co-evaporated with toluene (2×10 mL) and dried under reduced pressure. The mixture was then dissolved in dry CH_2Cl_2 (25 mL) in presence of 4Å molecular sieves and cooled at 0 °C. The $BF_3{\cdot}Et_2O$ (0.034 mL, 0.278 mmol) was added and the reaction allowed to warm to r.t. After 2.5 h, the reaction mixture was quenched with Et_3N, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (EtOAc-Et_2O, 3:2) to afford disaccharide **23** as a white solid (0.065 g, 68 %). R_f = 0.37 (EtOAc-Et_2O, 3:2). m.p.: 106-108 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.71 (d, *J*=10.0 Hz, 1H, NH), 5.21-5.10 (m, 2H, H-4', H-3), 5.01 (dd, *J*=10.5, 3.0 Hz, 1H, H-2'), 4.94 (t, *J*=9.1 Hz, 1H, H-3'), 4.64 (d, *J*=10.1 Hz, 1H, H-1), 4.56 (d, *J*=7.8 Hz, 1H, H-1'), 4.21-4.09 (m, 4H, H-2, H-4, H-6a, H-6a'), 3.93-3.87 (m, 2H, H-6b, H-6b'), 3.79-3.73 (m, 2H, H-5, CHCN), 3.65-3.60 (m, 1H, H-5'), 3.27 (d, *J*=17.3 Hz, 1H, CHCN), 1.96-2.14 (7s, 21H, OAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.55 (SCH_2CN), 20.39-29.57 ($7CH_3$), 52.70 (C-2), 61.38 (C-6), 67.14 (C-6'), 68.50 (C-5'), 68.81 (C-4),

68.85 (C-2'), 70.88, 70.89 (C-3', C-4'), 73.39 (C-3), 77.77 (C-5), 83.48 (C-1), 101.44 (C-1'), 116.86 (SCH_2CN), 169.56-171.40 (7C=O). ESI-HRMS (m/z) calcd for $C_{28}H_{38}N_2NaO_{16}S$ $[M+Na]^+$: 713.1840, found: 713.1859.

Cyanomethyl (2',3',4',6'-tetra-O-acetyl-α-D-mannopyranosyl)-(1→6)-2-acetamido 3,4-di-O-acetyl-2-deoxy-1-thio-β-D-glucopyranoside ***(24)***. 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose trichloroacetimidate (0.20 g, 0.417 mmol) and compound **14** (0.075 g, 0.208 mmol) were co-evaporated with toluene (2×10 mL) and dried under reduced pressure. The mixture was then dissolved in dry CH_2Cl_2 (25 mL) in presence of 4Å molecular sieves and cooled at 0 °C. The $BF_3{\cdot}Et_2O$ (0.058 mL, 0.417 mmol) was added and the reaction allowed to warm to r.t. After 2.5 h, the reaction mixture was quenched with Et_3N, filtered and concentrated *in vacuum*. The residue was purified by flash column chromatography (EtOAc-Et_2O, 3:2) to afford disaccharide **24** as a white solid (0.1 g, 70 %). R_f = 0.39 (EtOAc-Et_2O, 3:2). m.p.: 99-102 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.92 (d, *J*=9.6 Hz, 1H, NH), 5.31-5.01 (m, 5H, H-4', H-3', H-1', H-4, H-4', H-5'), 4.66 (d, *J*=10.6 Hz, 1H, H-1), 4.58 (dd, *J*=13.6, 2.1 Hz, 1H, H-3), 4.28-4.03 (m, 5H, H-2, H-2', H-6, Ha-6'), 3.86 (t, *J*=9.6 Hz, 1H, H-5), 3.71-3.65 (m, 2H, CHCN, Hb-6'), 3.30 (d, *J*=17.6 Hz, 1H, CHCN), 2.14, 2.13, 2.11, 2.09, 2.04, 1.98, 1.96 (7s, 21H, OAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.72 (SCH_2CN), 20.97-20.77 ($6CH_3$, OAc), 23.21 (CH_3, NAc), 52.96 (C-2), 62.33 (C-6, C'-6) , 62.62 (C'-2), 65.73, 68.67, 69.70, 70.13 (C-3, C'-3, C'-4, C'-5, C-4), 75.30 (C-5), 83.09 (C-1), 100.06 (C'-1), 116.17 (SCH_2CN), 169.71-171.80 (7C=O). ESI-HRMS (m/z) calcd for $C_{28}H_{38}N_2NaO_{16}S$ $[M+Na]^+$: 713.1840, found: 713.1828.

Cyanomethyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α/β-D-galacto-2-nonulopyranosylonate)-(2→6)-2-acetamido-3,4-di-O-acetyl-2-deoxy-β-D-glucopyranose ***(25)***. The sialyl xanthates (248 mg, 0.42 mmol) and compound **14** (100 mg, 0.28 mmol) were dissolved in the

CH_3CN (4.5 mL) stirred for 1 h with 4Å powdered molecular sieves. Then it cooled to -40 °C, and NIS (187 mg, 0.83 mmol) and TfOH (36 µL, 0.46 mmol) were added. After 2 h, the reaction was quenched with Et_3N. The mixture was diluted with CH_2Cl_2, filtered through celite, washed with 20% aqueous $Na_2S_2O_3$ solution, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column (Cy-EtOAc, 1:4) to obtain the disaccharide **25** (127 mg, 55%) as α and β isomer in the ratio of 3:2 from ^{1}H NMR. R_f = 0.35 (Cy-EtOAc 1:4, twice). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.63, 4.62, H-1'; 3.81, 3.79, $COOCH_3$; 3.51, 3,25, SCH_2CN; 2.64, 2.48, H-3eq; 2.20-1.89, OAc, NAc. ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.93-167.49, C=O; 115.18, CN; 98.42, 98.32, C-2; 83.53, 83.50, C-1'; 52.94, 52.77, $COOCH_3$; 51.12, C-2'; 49.33, C-5; 37.59, C-3; 23.22-20.61, NAc, OAc; 14.20, SCH_2CN. ESI-HRMS (m/z) calcd for $C_{34}H_{47}NO_{22}Na$ $[M+Na]^+$: 870.2579, found: 870.2571.

*O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α/β-D-galacto-2-nonulopyranosylonate)-(2→6)-2- acetamido-1,3,4-tri-O-acetyl-2-deoxy-α-D-glucopyranose (**26**).* The method in the literature[39] was used with some modifications: A mixture of sialyl xanthates (137 mg, 0.23 mmol) and compound **12** (40 mg, 0.12 mmol) with 4Å powdered molecular sieves were dissolved in the dry CH_3CN (2.4 mL) and CH_2Cl_2 (1.2 mL) stirring at r.t. for 1 h. Further, AgOTf (50 mg, 0.19 mmol) and DTBP (46 µL, 0.21 mmol) were added, and the mixture was cooled to -68 °C and kept protected from light. Then PhSCl (22 µL, 0.19 mmol) in the dry CH_2Cl_2 (0.12 mL) was added by running the solution down the cold wall of the reaction flask. Finally, the mixture was stirred for 2.5 h at -68 °C. After that, the mixture was diluted with a suspension of silica gel (0.5 g) in EtOAc (3 mL), filtered through celite, washed with saturated aqueous $NaHCO_3$ and water, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed (MeOH-EtOAc 1:20 to 1:5) to give disaccharide **26** (58 mg, 60%) as α and β isomer in the ratio of 2:1 from ^{1}H NMR. R_f = 0.29 (MeOH-EtOAc 1:10). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.22, 6.16, H-1'; 3.81, 3.79 $COOCH_3$; 2.63, 2.47, H-3eq; 2.20-2.05, OAc; 1.94-1.89 NAc. ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.93-167.49,

C=O; 98.42, 98.32, C-2; 90.82, 90.68, C-1'; 52.94, 52.77, $COOCH_3$; 51.21, 51.12, C-2'; 49.33, C-5; 37.59, C-3; 23.22-23.08, NAc; 21.18-20.61, OAc. ESI-HRMS (m/z) calcd for $C_{34}H_{47}NO_{22}Na$ $[M+Na]^+$: 857.2804, found: 857.2800.

O-*(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α/β-D-galacto-2-nonulopyranosylonate)-(2→6)-2-acetamido-1,3,4-tri-O-acetyl-2-deoxy-α-D-galactopyranose (**27**).* A mixture of sialyl xanthates (343 mg, 0.58 mmol) and compound **21** (40 mg, 0.29 mmol) with 4Å powdered molecular sieves were dissolved in the dry CH_3CN (6.0 mL) and CH_2Cl_2 (3.0 mL) stirring at r.t. for 1 h. Further, AgOTf (123 mg, 0.47 mmol) and DTBP (115 µL, 0.53 mmol) were added, and the mixture was cooled to -68 ºC and kept protected from light. Then PhSCl (54 µL, 0.47 mmol) in the dry CH_2Cl_2 (0.3 mL) was added by running the solution down the cold wall of the reaction flask. Finally, the mixture was stirred for 3 h at -68 ºC. After that, the mixture was diluted with a suspension of silica gel (1.25 g) in EtOAc (7.5 mL), filtered through celite, washed with saturated aqueous $NaHCO_3$ and water, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed (MeOH-EtOAc 0:1 to 1:9) to give disaccharide **27** (150 mg, 64%) as α and β isomer in the ratio of 1:1 from ^{1}H NMR. R_f = 0.26 (EtOAc, twice). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.18, H-1'; 3.78, 3.76 $COOCH_3$; 2.50, 2.43, H-3eq; 2.18-1.85, OAc, NAc. ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.06-167.15, C=O; 98.84, 98.58, C-2; 90.32, C-1'; 53.12, 52.03, $COOCH_3$; 49.33, C-2'; 48.00, C-5; 37.88, 36.57, C-3; 23.26-20.81, NAc, OAc. ESI-HRMS (m/z) calcd for $C_{34}H_{47}NO_{22}Na$ $[M+Na]^+$: 857.2804, found: 857.2815.

*O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-1,3,4-tri-O-acetyl-2-phthalimido-2-deoxy-α-D-glucopyranoside (**28**).* A mixture of sialyl xanthates (912 mg, 1.54 mmol) and acceptor **17** (370 mg, 0.85 mmol) with 4Å powdered molecular sieves were dissolved in the dry CH_3CN (17.2 mL) and CH_2Cl_2 (8.6 mL) stirring at r.t. for 1 h. Further, AgOTf (360 mg, 1.38 mmol) and DTBP (337 µL, 1.55 mmol) were added, and

the mixture was cooled to -68 °C and kept protected from light. Then PhSCl (337 µL, 1.38 mmol) in the dry CH_2Cl_2 (0.86 mL) was added by running the solution down the cold wall of the reaction flask. Finally, the mixture was stirred for 3 h at -68 °C. After that, the mixture was diluted with a suspension of silica gel (3.6 g) in EtOAc (22.5 mL), filtered through celite, washed with saturated aqueous $NaHCO_3$ and water, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed (Cy-EtOAc 1:3) to give disaccharide **28** (540 mg, 70%). R_f = 0.31 (Cy-EtOAc 1:3, twice). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84-7.81 (m, 2H, phthalimido protons), 7.73-7.71 (m, 2H, phthalimido protons), 6.52 (dd, *J*=9.2, 2.2 Hz, 1H, H-3'), 6.24 (d, *J*=3.9 Hz, 1H, H-1'), 5.35-5.15 (m, 3H, H-4', H-7, H-8), 4.89-4.82 (m, 1H, H-4), 4.68 (dd, *J*=8.2, 3.6 Hz, 1H, H-2'), 4.28-4.23 (m, 2H, H-5', H-9a), 4.03-3.99 (m, 4H, Ha-6', H-5, H-6, H-9b), 3.80 (s, 3H, $COOCH_3$), 3.40 (dd, *J*=11.4, 1.6 Hz, 1H, Hb-6'), 2.65 (dd, *J*=12.7, 4.4 Hz, 1H, H-3eq), 2.14 (s, 3H, OAc), 2.13 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.96 (t, *J*=3.6 Hz, 1H, H-3ax), 1.85 (s, 3H, NAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.17, 170.73, 170.40, 170.32, 170.22, 170.18, 169.83, 169.56, 169.52, 167.79 (C=O), 134.51, 123.81 (aromatic C), 98.46 (C-2), 90.80 (C-1'), 72.47, 70.74, 69.26, 69.14, 68.01, 67.50, 67.33 (C-6, C-5', C-4', C-4, C-8, C-3', C-7), 62.60 (C-9), 62.30 (C-6'), 53.67 (C-2'), 53.05 ($COOCH_3$), 49.50 (C-5), 37.89 (C-3), 23.33 (CH_3, NAc), 21.29, 21.15, 20.99, 20.96, 20.92, 20.85, 20.81 (CH_3, OAc). ESI-HRMS (m/z) calcd for $C_{34}H_{47}NO_{22}Na$ $[M+Na]^+$: 931.2596, found: 931.2590.

Methyl O-(methyl-5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-3,4-di-O-acetyl-2-phthalimido-2-deoxy-β-*D-glucopyranoside (**29**).* A mixture of sialyl xanthates (117 mg, 0.20 mmol) and acceptor **18** (40 mg, 0.10 mmol) with 4Å powdered molecular sieves were dissolved in the dry CH_3CN (1.66 mL) and CH_2Cl_2 (0.83 mL) stirring at r.t. for 1 h. Further, AgOTf (50 mg, 0.20 mmol) and DTBP (45 µL, 0.20 mmol) were added, and the mixture was cooled to -68 °C and kept protected from light. Then PhSCl (24 µL, 0.21 mmol) in the dry CH_2Cl_2 (0.13 mL) was added by running the solution down the cold wall of the reaction

flask. Finally, the mixture was stirred for 3 h at -68 °C. After that, the mixture was diluted with a suspension of silica gel (0.42 g) in EtOAc (2.5 mL), filtered through celite, washed with saturated aqueous $NaHCO_3$ and water, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed (Cy-EtOAc 1:3) to give disaccharide **29** (55 mg, 64%). R_f = 0.47 (Cy-EtOAc 1:3, twice). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88-7.86 (m, 2H, phthalimido protons), 7.76-7.74 (m, 2H, phthalimido protons), 5.78 (dd, *J*=9.1, 1.2 Hz, 1H, H-3'), 5.44 (d, *J*=10.9 Hz, 1H, H-1'), 5.32-5.17 (m, 3H, H-4', H-7, H-8), 4.94-4.85 (m, 1H, H-4), 4.68 (dd, *J*=9.2, 3.6 Hz, 1H, H-2'), 4.28-4.23 (m, 2H, H-5', H-9a), 4.06-4.01 (m, 4H, Ha-6', H-5, H-6, H-9b), 3.80 (s, 3H, $COOCH_3$), 3.40 (dd, *J*=11.4, 1.6 Hz, 1H, Hb-6'), 3.31 (s, 3H, OCH_3), 2.65 (dd, *J*=12.7, 4.4 Hz, 1H, H-3eq), 2.13 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.96 (t, *J*=3.6 Hz, 1H, H-3ax), 1.86 (s, 3H, NAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.17, 170.72, 170.39, 170.33, 170.22, 170.18, 169.83, 169.56, 169.51, 167.80 (C=O), 134.51, 123.81 (aromatic C), 98.46 (C-2), 91.90 (C-1'), 72.47, 70.74, 69.26, 69.14, 68.01, 67.50, 67.33 (C-6, C-5', C-4', C-4, C-8, C-3', C-7), 62.60 (C-9), 62.30 (C-6'), 60.53 (OCH_3), 53.67 ($COOCH_3$), 53.05 (C-2'), 49.50 (C-5), 37.89 (C-3), 23.33 (CH_3, NAc), 21.29, 21.15, 20.99, 20.96, 20.92, 20.85 (CH_3, OAc). ESI-HRMS (m/z) calcd for $C_{34}H_{47}NO_{22}Na$ $[M+Na]^+$: 903.2647, found: 903.2658.

Ethyl O-(methyl-5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-3,4-di-O-acetyl-2-phthalimido-2-deoxy-1-thio-β-*D-glucopyranoside **(30)**.* The method in the literature was used[40] with some modifications: The acceptor **19** (20 mg, 0.046 mmol) and acetochloroneuraminate methyl ester (70 mg, 0.138 mmol) in the dry CH_2Cl_2 (4.6 ml) were stirred with 4Å powdered molecular sieves for 1 h at r.t.. Then it was cooled to -45°C and kept protected from light, and AgOTf (83 mg, 0.322 mmol) was added. The mixture was gradually warmed to r.t. during 12 h, and further stirred at r.t. for 3 h. At last, filtered by celite and concentrated under reduced pressure. The residue was chromatographed (DCM-MeOH 9:1) to give disaccharide **30** (27 mg, 65%). R_f = 0.41 (Cy-EtOAc 1:3, twice). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88-7.85 (m, 2H,

phthalimido protons), 7.76-7.74 (m, 2H, phthalimido protons), 5.81 (dd, *J*=9.1, 1.2 Hz, 1H, H-3'), 5.45 (d, *J*=10.9 Hz, 1H, H-1'), 5.30-5.17 (m, 3H, H-4', H-7, H-8), 4.94-4.85 (m, 1H, H-4), 4.51 (dd, *J*=9.2, 3.6 Hz, 1H, H-2'), 4.36-4.30 (m, 2H, H-5', H-9a), 4.17-4.02 (m, 5H, H-6', H-5, H-6, H-9b), 3.80 (s, 3H, $COOCH_3$), 2.73-2.55 (m, 3H, H-3eq, SCH_2CH_3), 2.13 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.96 (t, *J*=3.6 Hz, 1H, H-3ax), 1.85 (s, 3H, NAc), 1.23 (t, *J*=1.9 Hz, 3H, SCH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.06, 172.00, 170.99, 170.56, 170.36, 170.33, 170.09, 170.06, 169.61, 167.61 (C=O), 134.66, 123.08 (aromatic C), 98.49 (C-2), 81.12 (C-1'), 76.19, 71.24, 70.15, 69.37, 68.96, 68.28, 67.34 (C-5', C-6, C-4', C-4, C-8, C-3', C-7), 62.24 (C-9), 62.07 (C-6'), 53.81 ($COOCH_3$), 51.89 (C-2'), 49.14 (C-5), 37.88 (C-3), 23.97 (SCH_2CH_3), 21.31 (CH_3, NAc), 19.34, 19.31, 19.29, 19.25, 19.21 (CH_3, OAc), 14.16 (SCH_2CH_3). ESI-HRMS (m/z) calcd for $C_{34}H_{47}NO_{22}Na$ $[M+Na]^+$: 933.2575, found: 933.2585.

Ethyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-3,4-di-O-acetyl-2-phthalimido-2-deoxy-1-thio-β-*D-galactopyranoside (**31**).* The acceptor **22** (20 mg, 0.046 mmol) and acetochloroneuraminate methyl ester (70 mg, 0.138 mmol) in the dry CH_2Cl_2 (4.6 ml) were stirred with 4Å powdered molecular sieves for 1 h at r.t.. Then it was cooled to -45 °C and kept protected from light, and AgOTf (83 mg, 0.322 mmol) was added. The mixture was gradually warmed to r.t. during 12 h, and further stirred at r.t. for 3 h. At last, filtered by celite and concentrated under reduced pressure. The residue was chromatographed (DCM-MeOH 9:1) to give disaccharide **31** (29 mg, 70%). R_f = 0.38 (Cy-EtOAc 1:3, twice). ^1H NMR (400 MHz, $CDCl_3$): δ 7.87-7.85 (m, 2H, phthalimido protons), 7.76-7.73 (m, 2H, phthalimido protons), 5.87 (dd, *J*=9.4, 2.9 Hz, 1H, H-3'), 5.72-5.70 (m, 2H, NH, H-4'), 5.52 (d, *J*=10.9 Hz, 1H, H-1'), 5.37-5.35 (m, 1H, H-8), 5.27-5.20 (m, 1H, H-4), 5.14-5.10 (m, 1H, H-7), 4.72 (dd, *J*=12.2, 3.6 Hz, 1H, H-6), 4.60 (t, *J*=10.2 Hz, 1H, H-2'), 4.19-4.05 (m, 3H, H-5, H-5', H-9a), 3.83 (s, 3H, $COOCH_3$), 3.66-3.59 (m, 2H, Ha-6', H-9b), 3.44 (t, *J*=7.2 Hz, 1H, Hb-6'), 2.74-2.63 (m, 1H, SCH_2CH_3), 2.47 (dd, *J*=12.7, 4.4 Hz, 1H, H-3eq), 2.35 (s, 3H, OAc), 2.16 (s, 3H, OAc), 2.11 (s,

3H, OAc), 2.04 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.89 (s, 3H, OAc), 1.86 (s, 3H, NAc), 1.83 (t, *J*=10.4 Hz, 1H, H-3ax), 1.20 (t, *J*=9.6 Hz, 3H, SCH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.13, 170.71, 170.67, 170.65, 170.43, 170.17, 169.56, 168.06, 167.61, 167.06 (C=O), 134.57, 134.40, 131.76, 131.39, 123.83 (aromatic C), 98.70 (C-2), 81.94 (C-1'), 75.10, 72.18, 72.08, 69.04, 69.01, 68.15, 67.70 (C-5', C-6, C-4, C-3', C-8, C-4', C-7), 62.50 (C-9), 60.55 (C-6'), 53.13 ($COOCH_3$), 50.76 (C-2'), 48.32 (C-5), 37.06 (C-3), 24.66 (SCH_2CH_3), 23.33 (CH_3, NAc), 21.43, 21.23, 21.07, 20.95, 20.65 (CH_3, OAc), 15.03 (CH_3). ESI-HRMS (m/z) calcd for $C_{34}H_{47}NO_{22}Na$ $[M+Na]^+$: 933.2575, found: 933.2580.

Synthesis and analysis of *neo*-glycoproteins

Neo-glycoproteins **23a** and **24a**, have been prepared according to the method previously reported[23] by activation of the disaccharides **23** and **24** (Figure S1), followed by glycosylation of the protein RNase A.

Synthesis of glycosphingolipid derivative 34

(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-1,3,4-tri-O-acetyl-2-phthalimido-2-deoxy-β-*D-glucopyranoside(1→1)-(2S,3R,4E)-2-azido-3-O-benzoyl-4-octadecene-l,3-diol-(**34**).* The $NH_2CH_2CH_2NH_2$ (18 uL) in the dry THF (5.2 mL) was added AcOH (18 uL) dropwise. Then compound **28** (200mg, 0.22 mmol) was added, and the mixture was further stirred at r.t. for 3 h. The solvent was evaporated and the residue was taken into CH_2Cl_2 and washed progressively with 1.0 M HCl, saturated aqueous $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$ and concentrated to give the crude intermediate **32** with the yield of 75%. Then the intermediate **32** (110 mg, 0.13 mmol) was dissolved in the dry CH_2Cl_2 (4.2 mL), cooled to -5 ^oC, and trichloroacetonitrile (420 µL) and 42 µL of DBU were added at -5 ^oC, and the mixture was stirred at -5 ^oC for 2 h. After concentration, the residue was purified by flash column chromatography (Cy-EtOAc 1:4) to yield the

trichloracetimidate **33** with α and β (4:1) mixture as pale yellow foam (77 mg, 60%). R_f = 0.45 (EtOAc). The **33** (60 mg, 0.059 mmol) and 3-*O*-benzoyl-azidosphingosine (50 mg, 0.12 mmol) in 2.8 mL of dry CH_2Cl_2 were stirred with 4Å powdered molecular sieves (500 mg) at r.t. for 1 h. The mixture was then cooled to -30 °C, and $BF_3{\cdot}Et_2O$ (60 μL, 0.48 mmol) was added dropwise, stirred for another 1.5 h at -30 °C, and then filtered through celite. Thefiltrate was washed with saturated aqueous $NaHCO_3$ and water, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was applied to a flash chromatography eluted with Cy-EtOAc 1:5 to give **34** (46 mg, 61%) as an amorphous solid. R_f = 0.58 (EtOAc). m.p.: 158-161 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98-7.95 (m, 2H, Ar-H), 7.86-7.82 (m, 2H, Ar-H), 7.73-7.71 (m, 2H, Ar-H), 7.57-7.53 (m, 1H, Ar-H), 7.44-7.40 (m, 2H, Ar-H), 5.83-5.64 (m, 1H, H-5''), 5.58-5.46 (m, 2H, H-3'', H-4''), 5.41-5.30 (m, 6H, H-8, H-7, H-5', H-3', NH, H-2'), 4.87-4.84 (m, 1H, H-4), 4.34-4.28 (m, 2H, H-1', Ha-9), 4.12-4.01 (m, 6H, Hb-9, H-5, H-6, H-2'', Ha-6', Ha-1''), 3.84-3.81 (m, 2H, Hb-1'', Hb-6'), 3.79 (s, 3H, $COOCH_3$), 3.58 (dd, *J*=5.7, 2.5 Hz, 1H, H-4'), 2.57 (dd, *J*=12.8, 4.6 Hz, 1H, H-3eq), 2.14 (t, *J*=1.5 Hz, 9H, 3OAc), 2.04 (s, 6H, 2OAc), 2.03 (d, *J*=2.1 Hz, 2H, H_2-6''), 2.02 (s, 3H, OAc), 1.93 (t, *J*=5.9 Hz, 1H, H-3ax), 1.87 (s, 3H, NAc), 1.24 (m, 22H, 11×CH_2), 0.87 (t, *J*=6.9 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.02 (C=O), 170.67 (C=O), 170.28 (C=O), 170.24 (C=O), 170.17 (2C=O), 170.15 (C=O), 170.03 (C=O), 168.55 (C=O), 167.70 (C=O), 164.87 (C=O), 138.92 (C-5''), 134.22 (C, CH aromatic), 133.13 (C, CH aromatic), 131.44 (C, CH aromatic), 129.70 (C, CH aromatic), 128.39 (C, CH aromatic), 123.60 (C-4''), 98.76 (C-1'), 98.49 (C-2), 74.78, 72.61, 72.44, 70.96, 69.13, 69.03, 68.58, 68.12, 67.37 (C-3'', C-3', C-4', C-6, C-4, C-5', C-1'', C-8, C-7), 63.35 (C-2''), 62.38 (C-6'), 60.79 (C-9), 54.46 (C-2'), 52.65 ($COOCH_3$), 49.51 (C-5), 38.19 (C-3), 31.91 (CH_2, C-6''), 29.68, 29.66, 29.64, 29.57, 29.39, 29.34, 29.15, 28.62 (CH_2), 23.20 (CH_3, NAc), 21.09 (CH_3, OAc), 20.85 (CH_3, OAc), 20.84 (CH_3, OAc), 20.82 (CH_3, OAc), 20.76 (CH_3, OAc), 20.70 (CH_3, OAc), 14.10 (CH_3). ESI-HRMS (m/z) calcd for $C_{57}H_{82}N_4O_{23}Na$ $[M+Na]^+$: 1300.5377, found: 1300.5365.

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[1] Y. Zhang, D. Lu, M. Sollogoub, Y. Zhang, Carbohydr. Chem. **2016**, *41*, 238-254.

[2] R. D. Astronomo, D. R. Burton, Nat. Rev. Drug Discov. **2010**, *9*, 308-324.

[3] H. Qu, J. Liu, J. Wdzieczak-Bakala, D. Lu, X. He, W. Sun, M. Sollogoub, Y. Zhang, Eur. J. Med. Chem. **2014**, *75*, 247-25.

[4] G. Källenius, A. Pawlowski, B. Hamasur, S. B. Svenson, Trends Microbiol. **2008**, *16*, 456-462.

[5] G. J. L. Bernardes, B. Castagner, P. H. Seeberger, ACS Chem. Biol. **2009**, *4*, 703-713.

[6] P. Stallforth, B. Lepenies, A. Adibekian, P. H. Seeberger, J. Med. Chem. **2009**, *52*, 5561–5577.

[7] O. Finco, R. Rappuoli, Front. Immunol. **2014**, *5*, 1-6.

[8] T. M. Altamore, C. Fernandez-Garcia, A. H. Gordon, T. Hubscher, N. Promsawan, M. G. Ryadnov, A. J. Doig, D. N. Woolfson, T. Gallagher, Angew. Chem. Int. Ed. **2011**, *50*, 11167-11171.

[9] S. Hakomori, Y. Zhang, Chemistry & Biology **1997**, *4*, 97-104.

[10] Y. Zhang, K. Iwabuchi, S. Nunomura, S. Hakomori, Biochemistry **2000**, *39*, 2459-2468.

[11] C. Zheng, M. Terreni, M. Sollogoub, Y. Zhang, Curr. Med. Chem. **2018**, *25*, 1-13 https://10.2174/0929867325666180129100619.

[12] Z. Yin, X. Huang, J. Carbohydr. Chem. **2012**, *31*, 143-186.

[13] T. Bavaro, L. Piubelli, M. Amicosante, M. Terreni, Curr. Org. Chem. **2016**, *20*, 1150-1168.

[14] E. W. Adams, D. M. Ratner, P. H. Seeberger, N. Hacohen, Chembiochem. **2018**, *9*, 294-303.

[15] R. M. Wilson, S. J. Danishefsky, J. Am. Chem. Soc. **2013**, *135*, 14462-14472.

[16] L. Cipolla, F. Peri, C. Airoldi, Anticancer Agents Med. Chem. **2008**, *8*, 92-121.

[17] Y. S. Cho, Q. Wan, S. J. Danishefsky, Bioorg. Med. Chem. **2015**, *13*, 5259-5266.

[18] S. I. van Kasteren, S. J. Campbell, S. Serres, D. C. Anthony, N. R. Sibson, B. G. Davis, Proc, Natl. Acad. Sci. USA. **2009**, *106*, 18-23.

[19] D. P. Gamblin, E. M. Scanlan, B. G. Davis, Chem. Rev. **2009**, *109*, 131-163.

[20] D. Lu, Y. Hu, M. Sollogoub, Y. Zhang, Carbohydr. Res. **2014**, *383*, 89-96.

[21] X. Huang, L. Huang, H. Wang, X. S. Ye, Angew. Chem. Int. Ed. **2004**, *43*, 5221-5224.

[22] M. Filice, J. M. Guisan, M. Terreni, J. M. Palomo, Nat. Protoc. **2012**, *7*, 1783-1796.

[23] T. Bavaro, M. Filice, C. Temporini, S. Tengattini, I. Serra, C. F. Morelli, G. Massolini, M. Terreni, RSC Adv. **2014**, *4*, 56455-56465.

[24] C. Temporini, T. Bavaro, S. Tengattini, I. Serra, G. Marrubini, E. Calleri, F. Fasanella, L. Piubelli, F. Marinelli, L. Pollegioni, G. Speranza, G. Massolini, M. Terreni, J. Chrom. A **2014**, *1367*, 57-67.

[25] C. Zheng, H. Qu, W. Liao, T. Bavaro, M. Terreni, M. Sollogoub, K. Ding, Y. Zhang, Eur. J. Med. Chem. **2018**, *146*, 613-620.

[26] T. Bavaro, M. Filice, P. Bonomi, Q. Abu Alassal, G. Speranza, J. M. Guisan, M. Terreni, Eur. J. Org. Chem. **2011**, 6181-6185.

[27] A. Rise Moen, T. Anthonsen, Biocatal. Biotransform. **2009**, *27*, 226-236.

[28] M. Filice, T. Bavaro, R. Fernandez-Lafuente, M. Pregnolato, J. M. Guisan, J. M. Palomo, M. Terreni, Catal. Today **2009**, *140*, 11-18.

[29] M. Filice, J. M. Palomo, P. Bonomi, T. Bavaro, R. Fernandez-Lafuente, J. M. Guisan, M. Terreni, Tetrahedron **2008**, *64*, 9286-9292.

[30] C. Navuluri, D. Crich, Angew. Chem. Int. Ed. **2013**, *52*, 11339-11342.

[31] S. Cai, B. Yu, Org. Lett. **2003**, *5*, 3827-3830.

[32] *S. Sahabuddin*, T. C. Chang, C .C. Lin, F. D. Jan, H. Y. Hsiao, K .T. Huang, J. H. Chen, J. C. Horng, J. A. Ho, Tetrahedron **2010**, *66*, 7510-7519.

[33] T. Bavaro, D. Ubiali, S. Brocca, S. Rocchietti, I. Nieto, M. Pregnolato, M. Lotti, M. Terreni, Biocatal. Biotransfor. **2010**, *28*, 108-116.

[34] P. Torres-Salas, A. Pedrali, T. Bavaro, S. Ambrosini, G. Marrubini, V. M. Pappalardo, G. Massolini, M. Terreni, D. Ubiali, Eur. J. Lipid Sci. Technol. **2014**, *116*, 1496-1504.

[35] A. Berkin, W. A. Szarek, R. Kisilevsky, Carbohydr. Res. **2002**, *337*, 37-44.

[36] Q. Abualassal, K. M. Al Azzam, J. A. Jilani, Biomed. Chromatogr. **2016**, *30*, 1416-1422.

[37] F. Yang, Y. Du, Carbohydr. Res. **2003**, *338*, 495-502.

[38] T. Peters, T. Weimar, Liebigs Annalen der Chemie **1991**, *3*, 237-242.

[39] V. Martichonok, G. M. Whitesides, J. Org. Chem. **1996**, *61*, 1702-1706.

[40] A. Lubineau, C. Augé, B. Bouxom, C. Gautheron, J. Carbohydr. Chem. **1992**, *11*, 59-70.

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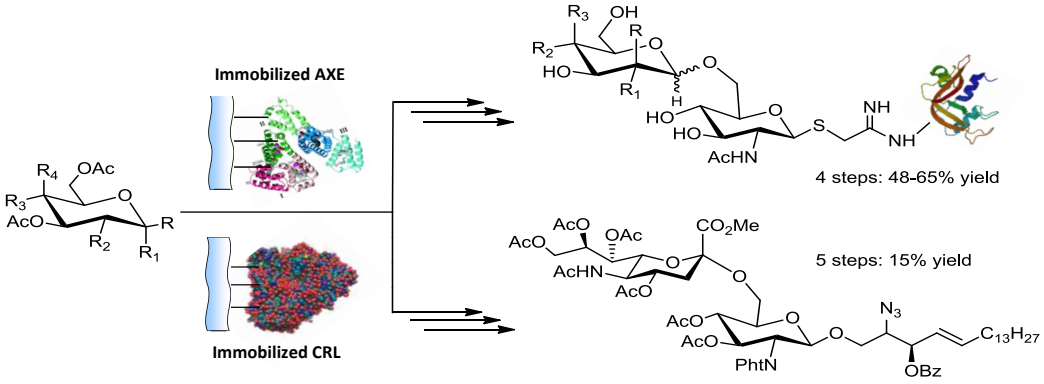
Entry for the Table of Contents



Chemoenzymatic approach, mediated by Acetyl xylan esterase (AXE) from *Bacillus pumilus* and *Candida rugosa* lipase (CRL), permits the concise synthesis of *neo*-glycoproteins and glycosphingolipids, respectively.