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αGal-conjugated anti-rhinovirus agents: chemo-enzymatic syntheses and testing of anti-Gal binding

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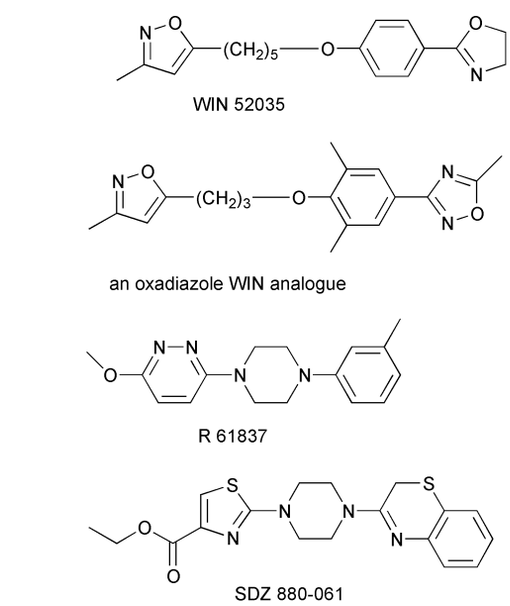
The syntheses of αGal-conjugated anti-rhinovirus agents **1**, **2** and **3** and their abilities to inhibit αGal binding of human anti-Gal antibody are described. An efficient enzymatic glycosylation using a novel fusion protein serves to provide the key αGal intermediate **7**, which is elaborated to αGal amines **9**, **12** and **14** with various tethers. The conjugates are then formed by amide coupling of these amines to heterocyclic acid **18** in the presence of 1,1′-carbonyldiimidazole (CDI), followed by deprotection of the αGal part. Conjugate **3** having a triethylene glycol linker displays the highest binding affinity to human anti-Gal antibody as tested by ELISA.

Introduction

Human rhinovirus[1] is a major cause of common colds, which result in worldwide morbidity and economic loss. Earlier attempts to develop a cross-protective vaccine have completely failed due to the diversity of rhinovirus serotypes (more than 100 identified). Therefore, efforts have been focused on the development of antiviral agents that can prevent the rhinovirus infection. The virion of rhinovirus consists of a non-enveloped capsid (a protein shell with some 'deep canyons') that surrounds a linear segment of a single-stranded RNA molecule.[2] Current anti-rhinovirus agents,[3] as represented by R 61837, WIN series, and SDZ series (Fig. 1), are categorised as capsid-binding molecules that exert their inhibitory effect by inserting themselves into the hydrophobic pocket of the virus and preventing the release of viral RNA. Although many structural modifications have been made to maximize their potency, these capsid-binding compounds are only effective in preventing the viral infection. In fact, none of the rhinovirus inhibitors have been found useful when given after symptoms of colds had started. Moreover, the sole dependence on certain antiviral agents to treat a virus infection will inevitably increase the possibility for the virus to develop resistance against the drugs.

As recently identified, the interaction between human anti-Gal antibody and αGal epitopes (carbohydrate structures bearing a Galα1-3Galβ terminus) is the mechanism responsible for the hyperacute rejection in xenotransplantation.[5] Trisaccharides Galα1-3Galβ1-4Glcβ-R (**A**), Galα1-3Galβ1-4GlcNAcβ-R′ (**B**), and pentasaccharide Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ-R″ (**C**) (Fig. 2) are the major αGal epitopes that are abundantly expressed on the cells of most mammals with the exception of humans, apes, and other Old World primates. Conversely, anti-Gal antibody, which interacts specifically with αGal epitopes, is the most abundant natural polyclonal antibody (including IgG, IgM and IgA) in humans. In hyperacute rejection, anti-Gal IgG binds to αGal epitopes on the xenograft cells and results in antibody-dependent cell-mediated cytotoxicity by human blood monocytes and macrophages. The IgM isotype of anti-Gal then fixes the complement and leads to complement-mediated lysis of the xenograft cells.[6] Indeed, this immunological rejection by the human body is so strong that pig organs transplanted into humans were hyperacutely rejected within minutes.

It is our hypothesis that anti-Gal-mediated human natural immune defense can be incorporated into the current antiviral strategy against rhinovirus (Fig. 3). By covalently attaching the αGal epitope to the capsid-binding molecules through appropriate linkers, a new generation of αGal-conjugated antiviral agents may be developed for more effective treatment



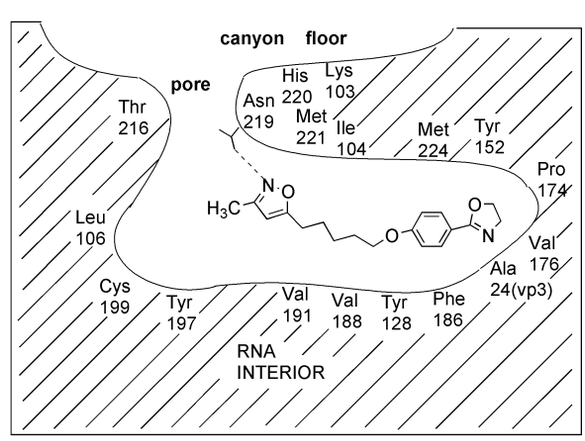


Fig. 1 Representative capsid-binding molecules and the binding of WIN 52035 in the pocket of HRV-14 with amino acid residues lining the wall.[4]

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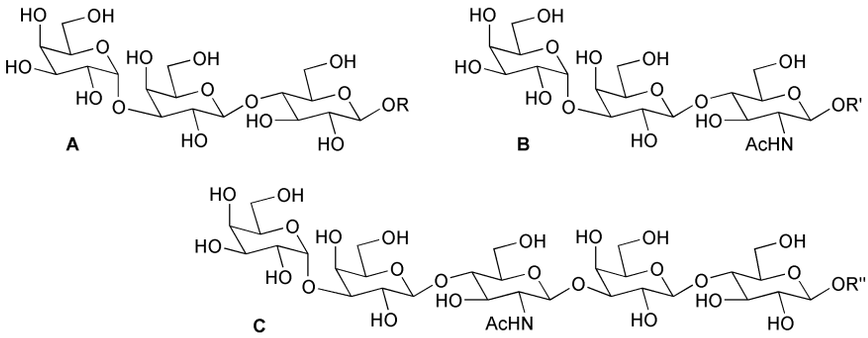


Fig. 2 Structures of the major αGal epitopes (**A**, **B** and **C**).

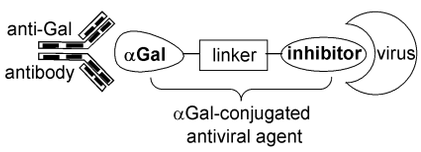
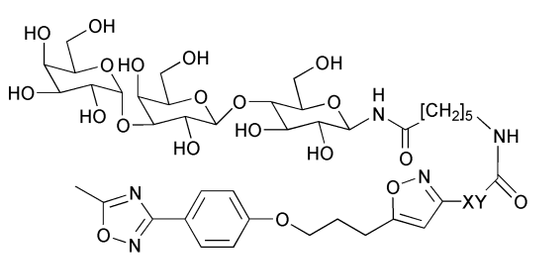


Fig. 3 Schematic representation of anti-Gal-mediated immune defense against rhinovirus through the αGal-conjugated antiviral agent.

of common colds. We envisage that the conjugate, once bound to the virus, will coat the virus with αGal epitopes, which will then induce an anti-Gal-mediated immune response and result in the killing of the virus. Herein, we report the chemoenzymatic synthesis of a series of such αGal-conjugated antiviral agents and their binding affinities to anti-Gal antibody.

Results and discussion

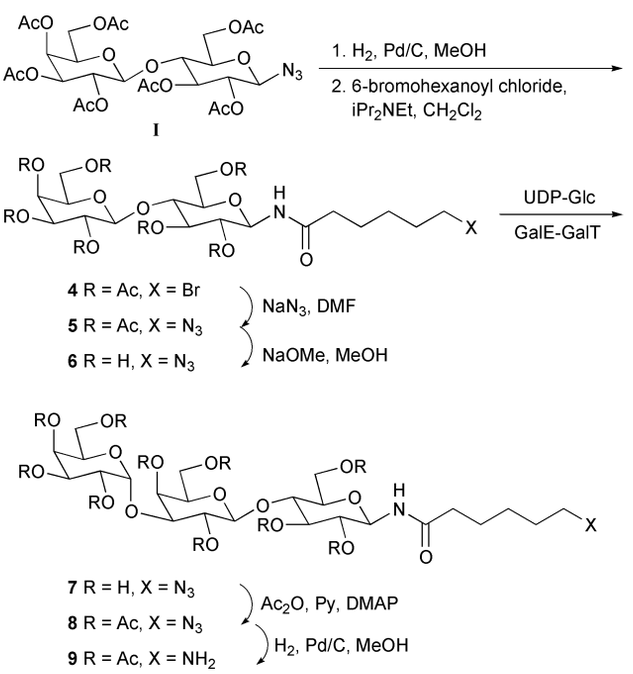
To allow for maximum retained binding ability of both parts in the conjugate, we have considered carefully the selection of all the building blocks, including the αGal epitope, the inhibitor, and the linker. For the αGal part, a type-**A** epitope (see Fig. 2) with an anomeric azide was chosen because of the ease of preparation and connection with linkers, as well as its strong binding to anti-Gal antibody. An oxadiazole WIN analogue (see Fig. 1), which is assumed to be more hydrolytically stable with comparable antiviral activity, was chosen as the inhibitor molecule. In order for the conjugate to be able to bind both anti-Gal antibody and the virus, it has to possess an appropriate linker between the two parts. This linker, with suitable length, flexibility and hydrophobicity, should then allow for the proper spatial arrangement of the conjugate so that both the αGal and the inhibitor can bind well.



1 XY = 0
2 XY = CONH[CH_2]$_5$
3 XY = CONH$CH_2CH_2OCH_2CH_2OCH_2CH_2$O

Synthesis of the key αGal intermediate 7 and elaboration to αGal amines 9, 12 and 14

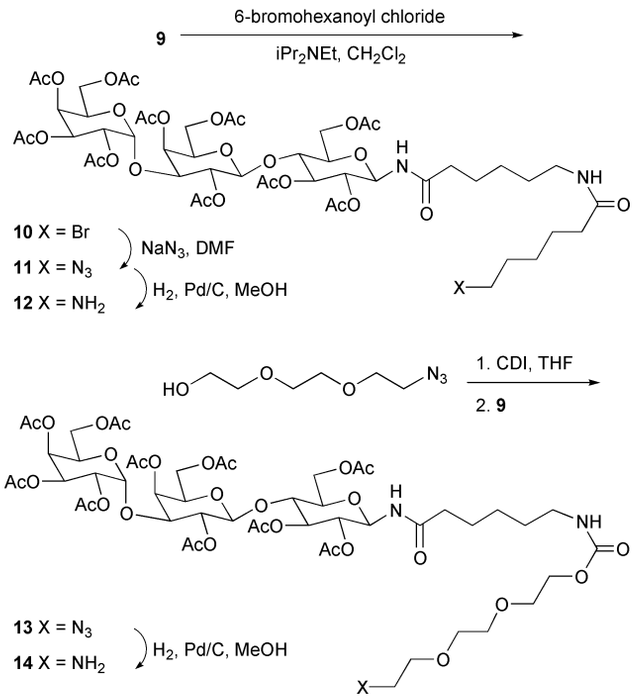
Although we have recently reported an efficient, large-scale chemical synthesis of a protected αGal trisaccharide derivative,[7] it still suffers from prerequisite protecting-group manipulations on the glycosyl acceptor. The enzymatic galactosylation method[8] was then pursued, which served as a key step in the synthesis of the αGal intermediate **7**, as illustrated in Scheme 1.



Scheme 1

Per-acetylated lactosyl azide[7] **I** was hydrogenated to give the primary amine, which was immediately treated with 6-bromohexanoyl chloride to afford **4**. Nucleophilic substitution of **4** with sodium azide in DMF afforded **5**, and subsequent deacetylation of **5** by sodium methoxide provided **6**. Compound **6** was subjected to enzymatic glycosylation with UDP-glucose (UDP = uridine 5′-diphosphate) as the donor and a fusion enzyme (GalE-GalT)[9] as the catalyst. Recently constructed in our laboratory, this enzyme has the dual functions of both UDP-Gal 4-epimerase (GalE) and α(1→3)-galactosyltransferase (GalT). As a result, it utilizes the relatively cheap glycosyl donor UDP-Glc in place of the expensive UDP-Gal. A gram-scale enzymatic glycosylation of **6** (0.96 g) was carried out, providing αGal trisaccharide **7** in 67% yield after purification by size-exclusion chromatography. Compound **7** was then per-acetylated to give **8**, and the azido group was reduced by catalytic hydrogenation to afford **9**.

To provide different linkers in the conjugate, compound **9** was elaborated to the other two αGal amines **12** and **14** according to Scheme 2. Prepared from **9** through a similar reaction sequence of acylation, substitution, and hydrogenation, compound **12** doubles the linker length by incorporating another 6-aminohexanoyl unit. A triethylene glycol spacer was introduced into compound **14** *via* a carbamate linkage using 1,1′-carbonyldiimidazole (CDI)[10] as the coupling agent. Thus,

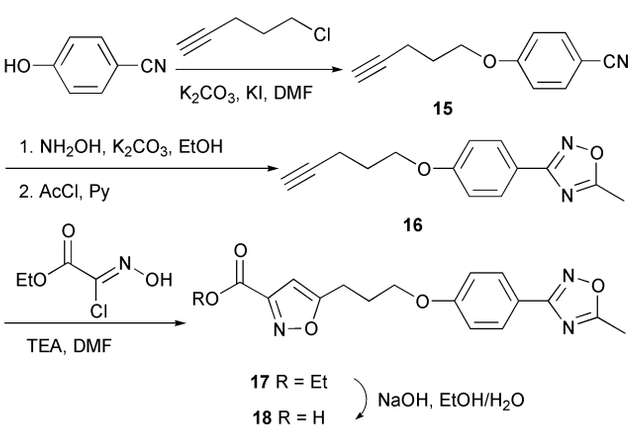


Scheme 2

activation of triethylene glycol monoazide[11] with CDI yielded the active intermediate, which then reacted with freshly made **9**† to give compound **13**. Hydrogenation of **13** then afforded the desired amine **14**.

Synthesis of the inhibitor and final couplings

The synthesis of the capsid-binding molecule is outlined in Scheme 3.[12] Reaction of 4-cyanophenol with 5-chloropent-1-



Scheme 3

yne gave nitrile **15**. Treatment of **15** with hydroxylamine yielded the intermediate amidoxime, which was acylated with acetyl chloride to give oxadiazole **16**. The desired isoxazole **17** was then obtained from a [3 + 2] cycloaddition reaction of **16** and the nitrile oxide derived from ethyl chlorooximidoacetate [ethyl chloro(hydroxyimino)acetate] and triethylamine. Subsequent basic hydrolysis of **17** afforded **18** with a terminal carboxylic acid ready to link to the αGal part.

The couplings of **18** with αGal amines **9**, **12** and **14** were again accomplished using CDI, which proved very effective and consistent for this system (Scheme 4). Thus, activation of **18** with CDI followed by addition of freshly made **9** (or **12**, **14**)

† All the amines (**9**, **12**, and **14**) with a per-acetylated αGal were prepared right before the coupling reaction to minimize the possibility of transesterification to the amino functionality.

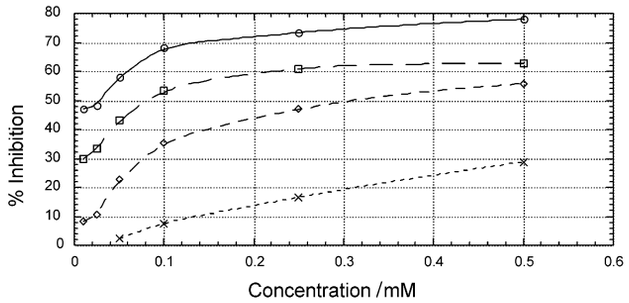


Fig. 4 Inhibition plot for compound **1** (×), **2** (◇), **3** (□), and a standard αGal epitope Galα1-3Galβ1-4GlcNAcβ-Allyl (○) as a reference.

provided per-acetylated conjugate **19** (or **20**, **21**) in moderate to good yields (47–65%). Final removal of the acetyl protecting groups under Zemplén conditions gave crude αGal conjugates **1**, **2** and **3**. The water-soluble compound **3** was purified by size-exclusion chromatography, while compounds **1** and **2** were purified by crystallization from boiling water.

Testing of the binding affinities of αGal conjugates 1, 2 and 3

The binding affinities of conjugates **1**, **2** and **3** to anti-Gal IgG were determined by inhibition ELISA[13] with purified human anti-Gal as the primary antibody and mouse laminin as a natural source of αGal. From the inhibition plot shown in Fig. 4, the concentrations of the conjugates at 50% inhibition (IC_{50}) of anti-Gal binding to αGal epitopes on mouse laminin were determined as follows: **1**, no inhibition up to 1 mM; **2**, 0.31 mM; and **3**, 0.08 mM.

These conjugates were compared with two known αGal trisaccharide standards, Galα1-3Galβ1-4GlcNAcβ-Allyl (IC_{50} 0.03 mM, used as a reference in the assay) and Galα1-3Galβ1-4Glcβ-NHAc (IC_{50} 0.07 mM, see ref. 8*a*). While compound **1** with the shortest linker fails to bind anti-Gal antibody up to 1 mM, compound **2**, having one more 6-aminohexanoyl unit, binds fairly well to the antibody. Interestingly, conjugate **3** with a triethylene glycol spacer is the best binder among the three, demonstrating a comparable anti-Gal binding affinity to the two αGal standards. The differences in the binding affinities of compound **1** on the one hand and compounds **2** and **3** on the other clearly reflect the influence of the linker between the αGal part and the inhibitor part. It seems that there is indeed a required spatial arrangement of the two parts, and that the longer and more flexible the linker, the better the binding affinity to anti-Gal.

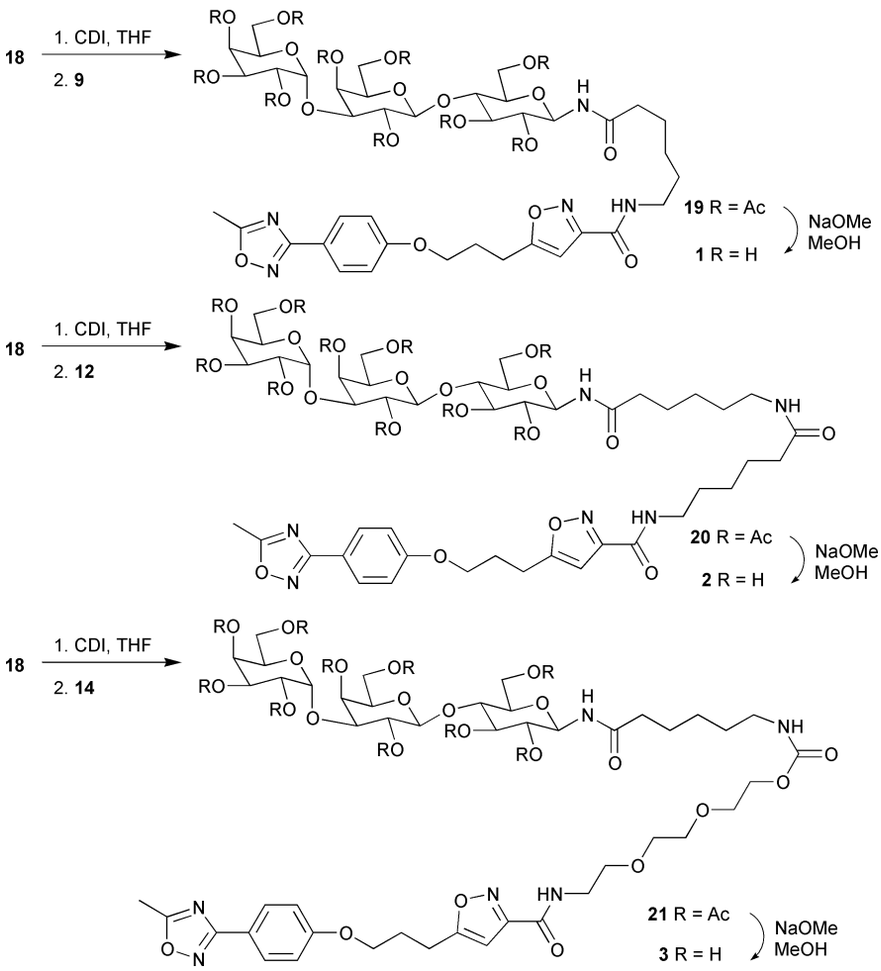
Conclusions

An efficient chemo-enzymatic synthesis of a series of αGal-conjugated antiviral agents has been developed and their binding affinities to anti-Gal antibody were established. It is hypothesized that these conjugates will not only prevent a virus infection, but also kill the virus through the action of human natural anti-Gal antibody, thus leading to more effective treatment of common colds. The promising anti-Gal binding ability of compound **3** will be explored for the design and synthesis of more such conjugates, and further work on evaluating the antiviral activity of these novel conjugates is underway.

Experimental

General

^1H and ^{13}C NMR spectra were recorded on 400 MHz Varian VXR400 and 500 MHz Varian Unity NMR spectrometers. Mass spectra were run at the mass spectrometry facility at Wayne State University. Baker silica gel (40 μm) was used for column chromatography, and E. Merck precoated TLC plates for TLC.



Scheme 4

6-Bromo-*N*-[*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-*β*-D-glucopyranosyl]hexanamide 4

A solution of per-acetylated *β*-D-lactosyl azide **I** (5.0 g, 7.6 mmol) in methanol (80 ml) was hydrogenated for 1.5 h at rt and 50 psi in the presence of Pd/C (0.6 g). The mixture was filtered through a pad of Celite. The filtrate was concentrated *in vacuo*, redissolved in dry CH_2Cl_2, and the solution was cooled to 0 °C. Diisopropylethylamine (3.9 ml, 22.8 mmol) and 6-bromohexanoyl chloride (2.3 ml, 15.2 mmol) were sequentially added, and the mixture was warmed to rt and stirred for 1 h. After concentration, the residue was dissolved in ethyl acetate, washed successively with dil. HCl, saturated aq. $NaHCO_3$, and brine, and dried (Na_2SO_4). The concentrated residue was purified by flash chromatography (ethyl acetate–hexanes 3 : 2) to give the title compound **4** (4.4 g, 72% over two steps) as a crystalline solid; δ_H(400 MHz; $CDCl_3$) 6.19 (d, J = 9.2 Hz, 1H), 5.31 (d, J = 2.8 Hz, 1H), 5.25 (t, J = 8.8 Hz, 1H), 5.16 (t, J = 9.4 Hz, 1H), 5.06 (dd, J = 10.4, 8.0 Hz, 1H), 4.90 (dd, J = 10.2, 3.4 Hz, 1H), 4.77 (t, J = 9.8 Hz, 1H), 4.42 (d, J = 8.0 Hz, 1H), 4.38 (m, 1H), 4.10 (m, 2H), 4.02 (dd, J = 11.2, 7.6 Hz, 1H), 3.84 (t, J = 7.0 Hz, 1H), 3.72 (m, 2H), 3.35 (t, J = 7.0 Hz, 2H), 2.15 (m, 2H), 2.11 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 2.00(3) (s, 3H), 2.00(0) (s, 3H), 1.92 (s, 3H), 1.81 (m, 2H), 1.57 (m, 2H), 1.39 (m, 2H); δ_C(100 MHz; $CDCl_3$) 173.0, 171.5, 170.6, 170.5, 170.4, 170.3, 169.5, 169.2, 101.1, 78.2, 76.2, 74.6, 72.5, 71.2, 71.1, 70.9, 69.1, 66.8, 62.1, 61.0, 36.4, 33.7, 32.5, 27.7, 24.3, 21.1, 21.0, 20.9(4), 20.8(7) (m), 20.8(3), 20.7.

6-Azido-*N*-[*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-*β*-D-glucopyranosyl]hexanamide 5

A mixture of **4** (4.4 g, 5.4 mmol) and sodium azide (0.53 g, 8.1 mmol) in DMF (20 ml) was heated at 70 °C for 12 h, and cooled to rt. After removal of DMF, the residue was dissolved in ethyl acetate, and washed twice with water, twice with dil. aq. NaCl, and once with brine, dried (Na_2SO_4), and concentrated to give the *title compound* **5** (4.1 g, 97%) as a slightly yellowish solid; δ_H(400 MHz; $CDCl_3$) 6.34 (br s, 1H), 5.29 (d, J = 3.2 Hz, 1H), 5.23 (t, J = 9.2 Hz, 1H), 5.16 (t, J = 9.4 Hz, 1H), 5.04 (dd, J = 10.2, 7.8 Hz, 1H), 4.89 (dd, J = 10.2, 3.4 Hz, 1H), 4.76 (t, J = 9.8 Hz, 1H), 4.42 (d, J = 8.0 Hz, 1H), 4.37 (m, 1H), 4.08 (m, 2H), 4.01 (dd, J = 11.0, 7.4 Hz, 1H), 3.83 (t, J = 6.6 Hz, 1H), 3.71 (m, 2H), 3.21 (t, J = 6.6 Hz, 2H), 2.12 (m, 2H), 2.10 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.98(4) (s, 3H), 1.98(0) (s, 3H), 1.90 (s, 3H), 1.55 (m, 4H), 1.31 (m, 2H); δ_C(100 MHz; $CDCl_3$) 173.1, 171.3, 170.5(3), 170.5(0), 170.3, 170.2, 169.5, 169.2, 101.1, 78.1, 76.2, 74.6, 72.6, 71.1(6), 71.1(4), 70.8, 69.2, 66.8, 62.2, 61.0, 51.3, 36.4, 28.7, 26.3, 24.7, 21.1, 21.0, 20.9, 20.8(4) (m), 20.8(0), 20.7; *m*/*z* (FAB MS) 775 (M^+ + H. $C_{32}H_{47}N_4O_{18}$ requires *m*/*z*, 775.29); 813 (M^+ + K. $C_{32}H_{46}N_4O_{18}$·K requires *m*/*z*, 813.24).

6-Azido-*N*-[*O*-(*β*-D-galactopyranosyl)-(1→4)-*β*-D-glucopyranosyl]hexanamide 6

To a solution of **5** (4.0 g, 5.2 mmol) in absolute methanol (200 ml) was added sodium methoxide to adjust pH to 9. The mixture was stirred at rt for 4 h and then neutralized with Dowex 50WX2-100 (H^+) resin. The resin was filtered off, and the filtrate was concentrated to give the *title compound* **6** (2.4 g, 97%) as a white solid; δ_H(500 MHz; D_2O) 4.81 (d, J = 9.0 Hz, 1H), 4.29 (d, J = 7.5 Hz, 1H), 3.76 (m, 2H), 3.65–3.49 (m, 8H), 3.39 (t, J = 8.5 Hz, 1H), 3.27 (m, 1H), 3.16 (t, J = 6.5 Hz, 2H), 2.18 (t, J = 7.0 Hz, 2H), 1.47 (m, 4H), 1.24 (m, 2H); δ_C(125 MHz; D_2O) 178.4, 103.0, 79.2, 77.9, 76.5, 75.5, 75.3, 72.6, 71.6, 71.1, 68.7, 61.2, 60.0, 51.1, 35.7, 27.8, 25.6, 24.7; *m*/*z* (FAB MS)

481 ($M^+ + H$. $C_{18}H_{33}N_4O_{11}$ requires *m*/*z*, 481.21); 519 ($M^+ + K$. $C_{18}H_{32}N_4O_{11}$·K requires *m*/*z*, 519.17).

6-Azido-*N*-[*O*-(*α*-D-galactopyranosyl)-(1→3)-*O*-(*β*-D-galactopyranosyl)-(1→4)-*β*-D-glucopyranosyl]hexanamide 7

To a mixture of **6** (0.96 g, 2.0 mmol), UDP-Glc (1.22 g, 2.0 mmol), bovine serum albumin (BSA) (0.1%), and $MnCl_2$ (10 mM) in Tris-HCl (100 mM, pH = 7.0; 40 ml) was added fusion enzyme GalE-GalT (10 U). The reaction mixture was shaken gently for 3 days at rt and then passed through a chloride-anion-exchange column (Dowex-Cl). The eluate was concentrated, and purified by gel-permeation chromatography on Bio-Gel P2 with doubly distilled water to give the *title compound* **7** (0.86 g, 67%) as a white solid; δ_H(500 MHz; D_2O) 4.97 (d, *J* = 3.5 Hz, 1H), 4.81 (d, *J* = 9.5 Hz, 1H), 4.36 (d, *J* = 8.0 Hz, 1H), 4.04–4.00 (m, 2H), 3.84 (m, 1H), 3.79–3.75 (m, 2H), 3.70–3.48 (m, 12H), 3.26 (t, *J* = 8.8 Hz, 1H), 3.15 (t, *J* = 6.8 Hz, 2H), 2.17 (t, *J* = 7.5 Hz, 2H), 1.46 (m, 4H), 1.23 (m, 2H); δ_C(125 MHz; D_2O) 178.5, 102.9, 95.5, 79.2, 78.1, 77.3, 76.5, 75.3, 75.2, 71.5, 70.9, 69.7, 69.4, 69.2, 68.3, 64.9, 61.1, 61.0, 60.0, 51.1, 35.7, 27.8, 25.5, 24.7; *m*/*z* (FAB MS) 643 ($M^+ + H$. $C_{24}H_{43}N_4O_{16}$ requires *m*/*z*, 643.27); 681 ($M^+ + K$. $C_{24}H_{42}N_4O_{16}$·K requires *m*/*z*, 681.22).

6-Azido-*N*-[*O*-(2,3,4,6-tetra-*O*-acetyl-*α*-D-galactopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-*β*-D-glucopyranosyl]hexanamide 8

A mixture of **7** (0.86 g, 1.34 mmol), pyridine (30 ml), acetic anhydride (20 ml), and 4-(dimethylamino)pyridine (DMAP) (20 mg) was stirred at rt for 3 h. After removal of pyridine and acetic anhydride, the residue was dissolved in ethyl acetate and washed successively with water, dil. HCl, saturated aq. $NaHCO_3$, and brine, and dried (Na_2SO_4). The concentrated residue was purified by flash chromatography with ethyl acetate–hexanes 3 : 1 to give the *title compound* **8** (1.26 g, 89%) as a crystalline solid; δ_H(400 MHz; $CDCl_3$) 6.17 (m, 1H), 5.41 (d, *J* = 2.0 Hz, 1H), 5.30–5.15 (m, 5H), 5.12 (dd, *J* = 10.2, 7.8 Hz, 1H), 5.05 (dd, *J* = 10.0, 3.2 Hz, 1H), 4.78 (t, *J* = 9.6 Hz, 1H), 4.36 (m, 2H), 4.17–3.98 (m, 6H), 3.81–3.71 (m, 4H), 3.23 (t, *J* = 7.0 Hz, 2H), 2.15 (m, 2H), 2.12 (s, 3H), 2.09 (s, 6H), 2.06 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.01(9) (s, 3H), 2.01(1) (s, 3H), 2.00 (s, 3H), 1.91 (s, 3H), 1.57 (m, 4H), 1.35 (m, 2H); δ_C(100 MHz; $CDCl_3$) 173.0, 171.4, 170.5(5), 170.4(5) (m), 170.3 (m), 170.0, 169.9, 169.4, 168.8, 101.0, 93.7, 78.2, 75.9, 74.7, 73.2, 72.5, 71.2, 71.0, 69.9, 67.9, 67.4, 67.1, 66.7, 64.9, 62.2, 61.4, 61.3, 51.4, 36.5, 28.7, 26.4, 24.7, 21.0, 20.9 (m), 20.8(5) (m), 20.7(8) (m), 20.6(6); *m*/*z* (FAB MS) 1063 ($M^+ + H$. $C_{44}H_{63}N_4O_{26}$ requires *m*/*z*, 1063.37); 1101 ($M^+ + K$. $C_{44}H_{62}N_4O_{26}$·K requires *m*/*z*, 1101.33).

4-(Pent-4-ynyloxy)benzonitrile 15

A mixture of 4-cyanophenol (0.99 g, 7.9 mmol), finely divided K_2CO_3 (2.19 g, 15.8 mmol), KI (0.132 g, 0.79 mmol), and 5-chloropent-1-yne (1.29 ml, 11.85 mmol) in DMF (15 ml) was stirred at 65 °C for 21 h. After cooling to room temperature, the mixture was partitioned between water and ethyl acetate. The aqueous layer was extracted twice with ethyl acetate. The combined organic phases were washed successively with water and brine, dried (Na_2SO_4), and concentrated. Flash chromatography of the residue with ethyl acetate–hexanes 1 : 3 provided the title compound **15** (1.53 g, 99%) as a white solid; δ_H(500 MHz; $CDCl_3$) 7.57 (d, *J* = 9.5 Hz, 2H), 6.95 (d, *J* = 9.0 Hz, 2H), 4.12 (t, *J* = 6.2 Hz, 2H), 2.41 (td, *J* = 6.9, 2.7 Hz, 2H), 2.02 (p, *J* = 6.6 Hz, 2H), 1.99 (t, *J* = 2.8 Hz, 1H); δ_C(125 MHz; $CDCl_3$) 162.9, 134.7, 119.9, 115.9, 104.6, 83.6, 70.0, 67.2, 28.5, 15.8.

5-Methyl-3-[4-(pent-4-ynyloxy)phenyl]-1,2,4-oxadiazole 16

A mixture of **15** (0.74 g, 4.0 mmol), finely divided K_2CO_3 (2.75 g, 20.0 mmol), and hydroxylamine hydrochloride (1.39 g, 20.0 mmol) in absolute ethanol (10 ml) was refluxed for 18 h. The hot mixture was filtered, and the remaining solids were washed with hot ethanol. The combined filtrates were concentrated *in vacuo*, and redissolved in pyridine (2.5 ml). Acetyl chloride (0.57 ml, 8.0 mmol) was added at a rate to maintain a gentle reflux. The mixture was refluxed for 2 h, cooled to room temperature, and filtered through a short column of silica gel. The filtrate was diluted with water, and extracted three times with CH_2Cl_2. The combined organic phases were washed successively with water and brine, dried (Na_2SO_4), and concentrated. Flash chromatography of the residue with ethyl acetate–hexanes 1 : 6 provided the *title compound* **16** (0.44 g, 45%) as a yellow oil which solidified upon storage; δ_H(500 MHz; $CDCl_3$) 7.98 (d, *J* = 8.5 Hz, 2H), 6.98 (d, *J* = 8.5 Hz, 2H), 4.12 (t, *J* = 6.0 Hz, 2H), 2.63 (s, 3H), 2.42 (td, *J* = 7.0, 2.5 Hz, 2H), 2.03 (p, *J* = 6.6 Hz, 2H), 1.99 (t, *J* = 2.8 Hz, 1H); δ_C(125 MHz; $CDCl_3$) 176.9, 168.8, 161.9, 129.6, 120.0, 115.4, 84.0, 69.7, 66.9, 28.7, 15.8, 13.1; *m*/*z* (HREIMS) 242.1052 (M^+. $C_{14}H_{14}N_2O_2$ requires *M*, 242.1055).

Ethyl 5-{3-[4-(5-methyl-1,2,4-oxadiazol-3-yl)phenoxy]propyl}-isoxazole-3-carboxylate 17

A solution of **16** (2.0 g, 8.26 mmol) in dry DMF (10 ml) was added dropwise over a period of 20 min to a solution of ethyl chlorooximidoacetate (3.75 g, 24.8 mmol) in DMF (25 ml). After being stirred at rt for 30 min, the solution was heated to 85 °C and a solution of TEA (3.45 ml, 24.8 mmol) in DMF (15 ml) was added dropwise over a period of 30 min. After an additional hour, the solution was cooled to rt, diluted with water, and extracted three times with ethyl acetate. The combined organic phases were washed successively with water, 10% aq. $NaHSO_4$, and brine, and dried (Na_2SO_4). The concentrated residue was purified by flash chromatography with ethyl acetate–hexanes 1 : 3 to afford the *title compound* **17** (1.83 g, 62%) as a white solid; δ_H(400 MHz; $CDCl_3$) 7.98 (d, *J* = 9.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.46 (s, 1H), 4.43 (q, *J* = 6.9 Hz, 2H), 4.07 (t, *J* = 6.2 Hz, 2H), 3.05 (t, *J* = 7.6 Hz, 2H), 2.63 (s, 3H), 2.24 (m, 2H), 1.41 (t, *J* = 7.2 Hz, 3H); δ_C(100 MHz; $CDCl_3$) 176.5, 174.6, 168.2, 161.1, 160.3, 156.7, 129.2, 119.8, 114.9, 102.1, 66.5, 62.3, 27.3, 23.7, 14.4, 12.6. *m*/*z* (HREIMS) 357.1325 (M^+. $C_{18}H_{19}N_3O_5$ requires *M*, 357.1325).

5-{3-[4-(5-Methyl-1,2,4-oxadiazol-3-yl)phenoxy]propyl}-isoxazole-3-carboxylic acid 18

A solution of **17** (145 mg, 0.41 mmol) and NaOH (20 mg, 0.5 mmol) in ethanol–water (1 : 1; 20 ml) was refluxed for 1 h, cooled to rt, and the ethanol was removed *in vacuo*. The aqueous solution was washed with diethyl ether, and AcOH was added. The chilled mixture was filtered, and the solids obtained were washed with water and dried (Na_2SO_4) *in vacuo* to give the title compound **18** (128 mg, 96%) as a white solid; δ_H(500 MHz; $CDCl_3$) 7.99 (d, *J* = 9.0 Hz, 2H), 6.97 (d, *J* = 9.0 Hz, 2H), 6.52 (s, 1H), 4.09 (t, *J* = 5.8 Hz, 2H), 3.08 (t, *J* = 7.5 Hz, 2H), 2.66 (s, 3H), 2.26 (m, 2H); δ_C(125 MHz; $CDCl_3$) 177.2, 175.6, 168.6, 162.8, 161.6, 156.7, 129.7, 120.1, 115.4, 102.8, 67.0, 27.7, 24.2, 13.1.

General procedure for catalytic hydrogenation for the preparation of amines 9, 12, and 14

A solution of the azido compound in methanol was hydrogenated for 1 h at rt and 50 psi in the presence of Pd/C (15 wt%). The mixture was filtered through a pad of Celite, and the filtrate was concentrated to give the amine, which was immediately used in the coupling reaction that follows.

General procedure for CDI-promoted coupling reactions for the synthesis of compounds 13, 19–21

A solution of the alcohol or the acid (2.2 eq.) and CDI (2.4 eq.)

in THF was refluxed for 2 h. After the solution had cooled to rt, freshly made amine (1.0 eq.) was added and reflux was resumed for 22 h. The solution was cooled to rt and concentrated *in vacuo*. The resultant yellow oil was partitioned between water and ethyl acetate; the organic phase was dried (Na_2SO_4) and concentrated. Flash chromatography of the residue with CH_2Cl_2–MeOH 30 : 1 afforded the product.

Compound 13

From triethylene glycol monoazide (40 mg, 0.22 mmol), CDI (40 mg, 0.24 mmol), THF (10 ml), and **9** (freshly prepared from 106 mg, 0.1 mmol of **8**) was obtained *compound* **13** (82 mg, 66%) as a white solid; δ_H(500 MHz; $CDCl_3$) 6.17 (d, J = 9.0 Hz, 1H), 5.43 (d, J = 2.0 Hz, 1H), 5.32–5.21 (m, 4H), 5.19 (t, J = 9.2 Hz, 1H), 5.14 (dd, J = 10.8, 7.8 Hz, 1H), 5.07 (dd, J = 10.8, 3.2 Hz, 1H), 4.84 (m, 1H), 4.80 (t, J = 9.5 Hz, 1H), 4.39–4.36 (m, 2H), 4.20–4.00 (m, 8H), 3.82–3.60 (m, 14H), 3.38 (t, J = 5.0 Hz, 2H), 3.13 (q, J = 6.5 Hz, 2H), 2.14 (s, 3H), 2.12 (s, 6H), 2.08 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.93 (s, 3H), 1.58 (m, 2H), 1.47 (m, 2H), 1.29 (m, 2H); δ_C(125 MHz; $CDCl_3$) 173.2, 171.5, 170.6, 170.5 (m), 170.4 (m), 170.1, 170.0, 169.5, 168.8, 156.7, 101.0, 93.7, 78.2, 75.9, 74.7, 73.2, 72.5, 71.2, 71.0, 70.9, 70.8, 70.3, 69.9, 69.8, 67.9, 67.4, 67.0, 66.7, 64.9, 64.0, 62.1, 61.4, 61.3, 50.9, 40.9, 36.6, 29.8, 26.3, 24.8, 21.1, 21.0 (m), 20.9 (m), 20.8(4) (m), 20.7(8), 20.7(1); *m*/*z* (FAB MS) 1238 (M^+ + H. $C_{51}H_{76}N_5O_{30}$ requires *m*/*z*, 1238.46); 1276 (M^+ + K. $C_{51}H_{75}N_5O_{30}$·K requires *m*/*z*, 1276.41).

Per-acetylated αGal conjugate 19

From **18** (74 mg, 0.22 mmol), CDI (40 mg, 0.24 mmol), THF (10 ml) and **9** (freshly prepared from 106 mg, 0.1 mmol of **8**) was obtained *compound* **19** (63 mg, 47%) as a white solid; δ_H(500 MHz; $CDCl_3$) 7.98 (d, J = 8.5 Hz, 2H), 6.96 (d, J = 9.0 Hz, 2H), 6.84 (t, J = 5.8 Hz, 1H), 6.49 (s, 1H), 6.16 (d, J = 9.0 Hz, 1H), 5.44 (m, 1H), 5.32–5.23 (m, 4H), 5.20 (t, J = 9.8 Hz, 1H), 5.15 (dd, J = 10.2, 8.2 Hz, 1H), 5.09 (dd, J = 10.8, 3.2 Hz, 1H), 4.81 (t, J = 9.5 Hz, 1H), 4.36 (m, 2H), 4.21–4.02 (m, 8H), 3.81 (dd, J = 10.0, 3.0 Hz, 1H), 3.78 (t, J = 6.8 Hz, 1H), 3.74 (m, 2H), 3.41 (q, J = 6.8 Hz, 2H), 3.04 (t, J = 7.5 Hz, 2H), 2.63 (s, 3H), 2.23 (m, 2H), 2.15 (s, 3H), 2.13 (s, 6H), 2.09 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.94 (s, 3H), 1.62 (m, 6H), 1.36 (m, 2H); δ_C(125 MHz; $CDCl_3$) 176.6, 174.5, 173.1, 171.6, 170.6, 170.5 (m), 170.4 (m), 170.1, 170.0, 169.5, 168.9, 168.2, 161.1, 159.3, 158.9, 129.2, 119.8, 114.9, 101.3, 101.1, 93.7, 78.2, 75.9, 74.7, 73.2, 72.5, 71.2, 71.0, 69.8, 67.9, 67.4, 67.0, 66.7, 66.5, 64.9, 62.1, 61.4, 61.3, 39.3, 36.5, 29.3, 27.3, 26.4, 24.8, 23.7, 21.1, 20.9(9) (m), 20.9(5) (m), 20.8(5) (m), 20.8, 20.7, 12.6; *m*/*z* (FAB MS) 1347.4 (M^+. $C_{60}H_{77}N_5O_{30}$ requires *M*, 1347.47); 1385.0 (M^+ − H + K. $C_{60}H_{76}N_5O_{30}$·K requires *m*/*z*, 1385.42).

Per-acetylated αGal conjugate 20

From **8** (106 mg, 0.1 mmol) following the sequence of hydrogenation (to **9**), acylation (to **10**) and azide substitution was obtained compound **11** (90 mg, 77%). From **18** (63 mg, 0.19 mmol), CDI (34 mg, 0.21 mmol), THF (10 ml) and **12** (freshly prepared from 90 mg, 0.077 mmol of **11**) was obtained *compound* **20** (65 mg, 58%) as a white solid; δ_H(500 MHz; $CDCl_3$) 7.98 (d, J = 8.5 Hz, 2H), 6.95 (d, J = 8.5 Hz, 2H), 6.91 (t, J = 5.8 Hz, 1H), 6.49 (s, 1H), 6.28 (d, J = 9.5 Hz, 1H), 5.65 (t, J = 5.8 Hz, 1H), 5.44 (m, 1H), 5.32–5.18 (m, 5H), 5.15 (dd, J = 10.2, 7.8 Hz, 1H), 5.08 (dd, J = 10.5, 3.5 Hz, 1H), 4.81 (t, J = 9.5 Hz, 1H), 4.39 (m, 2H), 4.20–4.01 (m, 8H), 3.81 (dd, J = 10.0, 3.0 Hz, 1H), 3.78 (t, J = 7.2 Hz, 1H), 3.74 (m, 2H), 3.42 (q, J = 6.8 Hz, 2H), 3.21 (q, J = 6.5 Hz, 2H), 3.03 (t, J = 7.8 Hz, 2H), 2.63 (s, 3H), 2.22 (m, 2H), 2.15 (s, 3H), 2.12(2) (s, 3H), 2.11(8) (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.04(4) (s, 3H), 2.03(7) (s, 3H), 2.02 (s, 3H), 1.94 (s, 3H), 1.78 (m, 2H), 1.69–1.57 (m, 8H), 1.47 (m, 2H), 1.38 (m, 2H), 1.28 (m, 2H); δ_C(125 MHz; $CDCl_3$) 176.5, 174.5, 173.3, 173.0, 171.5, 170.5 (m), 170.4 (m), 170.1, 170.0, 169.5, 168.9, 168.2, 161.1, 159.3, 159.0, 129.2, 119.7, 114.9, 101.3, 101.1, 93.7, 78.1, 75.9, 74.7, 73.1, 72.5, 71.2, 71.0, 69.8, 67.9, 67.4, 67.0, 66.7, 66.6, 64.9, 62.1, 61.4, 61.3, 39.5, 39.3, 36.7, 36.5, 29.4(3), 29.3(9), 27.3, 26.6, 26.4, 25.4, 24.8, 23.7, 21.1, 20.9(9) (m), 20.9(5) (m), 20.8(5) (m), 20.8, 20.7, 12.6; *m*/*z* (FAB MS) 1461.4 (M^+ + H. $C_{66}H_{89}N_6O_{31}$ requires *m*/*z*, 1461.56); 1499.4 (M^+ + K. $C_{66}H_{88}N_6O_{31}$·K requires *m*/*z*, 1499.51).

Per-acetylated αGal conjugate 21

From **18** (65 mg, 0.20 mmol), CDI (35 mg, 0.22 mmol), THF (10 ml) and **14** (freshly prepared from 82 mg, 0.066 mmol of **13**) was obtained *compound* **21** (66 mg, 65%) as a white solid; δ_H(500 MHz; $CDCl_3$) 7.98 (d, J = 8.5 Hz, 2H), 7.24 (m, 1H), 6.96 (d, J = 9.0 Hz, 2H), 6.48 (s, 1H), 6.21 (d, J = 10.0 Hz, 1H), 5.44 (m, 1H), 5.32–5.18 (m, 5H), 5.15 (dd, J = 10.2, 8.2 Hz, 1H), 5.08 (dd, J = 10.5, 3.0 Hz, 1H), 5.03 (m, 1H), 4.81 (t, J = 9.5 Hz, 1H), 4.39–4.36 (m, 2H), 4.19–4.01 (m, 10H), 3.82–3.62 (m, 16H), 3.13 (q, J = 6.7 Hz, 2H), 3.03 (t, J = 7.8 Hz, 2H), 2.63 (s, 3H), 2.22 (m, 2H), 2.15 (s, 3H), 2.12 (s, 6H), 2.08 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.04(5) (s, 3H), 2.03(6) (s, 3H), 2.02 (s, 3H), 1.94 (s, 3H), 1.58 (m, 2H), 1.47 (m, 2H), 1.28 (m, 2H); δ_C(125 MHz; $CDCl_3$) 176.5, 174.4, 173.2, 171.5, 170.6, 170.5 (m), 170.4 (m), 170.1, 170.0, 169.5, 168.8, 168.2, 161.1, 159.4, 158.9, 156.7, 129.2, 119.8, 114.9, 101.3, 101.0, 93.7, 78.2, 75.9, 74.7, 73.2, 72.5, 71.2, 71.0, 70.7, 70.5, 69.9, 69.8 (m), 67.9, 67.4, 67.0, 66.7, 66.5, 64.9, 64.0, 62.2, 61.4, 61.3, 40.9, 39.5, 36.6, 29.8, 27.3, 26.4, 24.9, 23.7, 21.1, 21.0 (m), 20.9 (m), 20.8(5) (m), 20.7(9), 20.7(1), 12.6; *m*/*z* (FAB MS) 1561 (M^+ + K. $C_{67}H_{90}N_6O_{34}$·K requires *m*/*z*, 1561.51).

αGal conjugate 1

To a solution of **19** (63 mg, 0.047 mmol) in absolute methanol (20 ml) at 0 °C was added sodium methoxide to adjust pH to 9. The mixture was stirred for 6 h and then neutralized with Dowex 50WX2-100 (H^+) resin. The resin was filtered off, and the filtrate was concentrated. Crystallization of the resultant residue from boiling water afforded *compound* **1** (32 mg, 74%) as a white solid; δ_H(500 MHz; CD_3CN–D_2O 1 : 1) 8.22 (d, J = 8.5 Hz, 2H), 7.35 (d, J = 9.0 Hz, 2H), 6.81 (s, 1H), 5.35 (d, J = 4.0 Hz, 1H), 5.18 (d, J = 9.0 Hz, 1H), 4.73 (d, J = 7.5 Hz, 1H), 4.43–4.37 (m, 4H), 4.22–4.20 (m, 1H), 4.16–4.12 (m, 2H), 4.08–3.85 (m, 11H), 3.66–3.58 (m, 4H), 3.31 (t, J = 7.8 Hz, 2H), 2.91 (s, 3H), 2.55 (t, J = 7.2 Hz, 2H), 2.49 (m, 2H), 2.29 (p, J = 2.4 Hz, CH_3CN), 1.88 (m, 4H), 1.64 (m, 2H); *m*/*z* (FAB MS) 950 (M^+ + Na. $C_{40}H_{57}N_5O_{20}$·Na requires *m*/*z*, 950.35).

αGal conjugate 2

To a solution of **20** (65 mg, 0.045 mmol) in absolute methanol (20 ml) at 0 °C was added sodium methoxide to adjust pH to 9. The mixture was stirred for 6 h and then neutralized with Dowex 50WX2-100 (H^+) resin. The resin was filtered off, and the filtrate was concentrated. Crystallization of the resultant residue from boiling water afforded *compound* **2** (34 mg, 73%) as a slightly yellowish solid; δ_H(500 MHz; CD_3CN–D_2O 1 : 1) 8.18 (d, J = 9.0 Hz, 2H), 7.31 (d, J = 8.5 Hz, 2H), 6.78 (s, 1H), 5.32 (d, J = 3.5 Hz, 1H), 5.15 (d, J = 10.0 Hz, 1H), 4.70 (d, J = 7.5 Hz, 1H), 4.40–4.33 (m, 4H), 4.19 (m, 1H), 4.13–4.09 (m, 2H), 4.06–3.82 (m, 11H), 3.63–3.57 (m, 4H), 3.35 (t, J = 7.0 Hz, 2H), 3.28 (t, J = 7.2 Hz, 2H), 2.88 (s, 3H), 2.50 (t, J = 7.0 Hz, 2H), 2.46 (t, J = 6.5 Hz, 2H), 2.42 (t, J = 7.8 Hz, 2H), 2.27 (p, J = 2.5 Hz, CH_3CN), 1.82 (m, 6H), 1.69 (m, 2H), 1.55 (m, 4H); *m*/*z* (FAB MS) 1041 (M^+ + H. $C_{46}H_{69}N_6O_{21}$ requires *m*/*z*, 1041.45); 1063 (M^+ + Na. $C_{46}H_{68}N_6O_{21}$·Na requires *m*/*z*, 1063.43); 1079 (M^+ + K. $C_{46}H_{68}N_6O_{21}$·K requires *m*/*z*, 1079.41).

αGal conjugate 3

To a solution of **21** (66 mg, 0.043 mmol) in absolute methanol (20 ml) at 0 °C was added sodium methoxide to adjust pH to 9. The mixture was stirred for 6 h and then neutralized with Dowex 50WX2-100 (H^+) resin. The resin was filtered off, and the filtrate was concentrated. The resultant residue was purified with gel-permeation chromatography (Sephadex G10) to afford *compound* **3** (36 mg, 75%) as a white solid; δ_H(500 MHz; CD_3CN–D_2O 1 : 9) 7.91 (d, J = 8.5 Hz, 2H), 7.06 (d, J = 8.5 Hz, 2H), 6.55 (s, 1H), 5.10 (s, 1H), 4.93 (d, J = 9.0 Hz, 1H), 4.48 (d, J = 7.5 Hz, 1H), 4.15–4.10 (m, 6H), 3.98–3.56 (m, 27H), 3.39 (m, 1H), 3.04 (q, J = 7.0 Hz, 4H), 2.65 (s, 3H), 2.26 (m, 2H), 1.56 (m, 2H), 1.43 (m, 2H), 1.28 (m, 2H); *m*/*z* (FAB MS) 1125.43 (M^+ + Na. $C_{47}H_{70}N_6O_{24}$·Na requires *m*/*z*, 1125.4339); 1141.41 (M^+ + K. $C_{47}H_{70}N_6O_{24}$·K requires *m*/*z*, 1141.4079).

Inhibition ELISA

An ELISA (enzyme-linked immunosorbent assay) was conducted using mouse laminin, a basement membrane glycoprotein containing 50–70 αGal epitopes per molecule, as the solid-phase antigen. Purified human (male, blood type AB) polyclonal anti-Gal antibody (32 μg ml^{-1}) or human sera (4-fold dilution) was first incubated with varying concentrations of αGal compounds for 3 h at room temperature. An aliquot (50 μL) of the mixture was then added to each microtiter plate well precoated with mouse laminin (50 μL $well^{-1}$ of 10 μg ml^{-1} in 0.05 M Na_2CO_3–$NaHCO_3$ buffer, pH = 9.5). After incubation for 1.5 h at room temperature, unbound antibodies were washed out with PBS–Tween buffer (pH = 7.4, 0.05% Tween, 5 × 200 μL $well^{-1}$). A secondary antibody (1 : 1000 peroxidase-conjugated goat anti-human IgG, 50 μL $well^{-1}$) was introduced, and the incubation was allowed to proceed for 1 h at room temperature. After washing of the mixture with PBS–Tween buffer (5 × 200 μL $well^{-1}$), standard substrate (3,3′,5,5′-tetramethylbenzidine–H_2O_2 9 : 1, 100 μL $well^{-1}$) was added. The enzymatic oxidation reaction produced a blue stain in each well. The staining was quenched by adding 0.1 M H_2SO_4 (100 μL $well^{-1}$). Readings of optical absorption were taken at 450 nm (Bio-Rad Microplate Reader, model 3550-UV). PBS with secondary antibody was used as a background control, and purified anti-Gal or human sera with secondary antibody as the maximum staining (*i.e.*, 0% inhibition). The % inhibition at each concentration was calculated as follows: $(M - S)/(M - B) = \%$ inhibition, where M was the OD_{450} reading of the maximum staining (mouse laminin + purified anti-Gal or human sera + 2nd Ab), S was the OD_{450} reading of the sample staining (mouse laminin + αGal conjugate + purified anti-Gal or human sera + 2nd Ab), and B was the OD_{450} reading of the background staining (mouse laminin + 2nd Ab).

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References

1 For a detailed review on rhinoviruses, see: W. Al-Nakib, in *Principles and Practice of Clinical Virology*, ed. A. J. Zuckerman, J. E. Banatvala and J. R. Pattison, Wiley, Chichester, 3rd edn., 1994.
2 M. R. Macnaughton, *Curr. Top. Microbiol. Immunol.*, 1982, **97**, 1.
3 For reviews on anti-rhinovirus agents, see: (*a*) *Antiviral Chemotherapy*, ed. D. J. Jefferies and E. De Clercq, Wiley, Chichester, 1992; (*b*) S. Kramczyk and N. Bischofberger, *Annu. Rep. Med. Chem.*, 1997, **32**, 141.
4 R. P. Rother, W. L. Fodor, J. P. Springhom, C. W. Birks, E. Setter, M. S. Sandrin, S. P. Squinto and S. A. Rollins, *J. Exp. Med.*, 1995, **182**, 1345.
5 (*a*) I. M. McMorrow, C. A. Comrack, D. H. Sachs and H. DerSimonian, *Transplantation*, 1997, **64**, 501; (*b*) F. Neethling, D. Joziasse, N. Bovin, D. K. C. Cooper and R. Oriol, *Transplant Int.*, 1996, **9**, 98; (*c*) D. K. C. Cooper, A. H. Good, E. Koren, R. Oriol, A. J. Malcolm, R. M. Ippolito, F. A. Neethling, Y. Ye, E. Romano and N. Zuhdi, *Transplant Immunol.*, 1993, **1**, 198; (*d*) J. L. Platt, *Nature*, 1998, April, supplement to vol. **392**, 11; (*e*) *Nature* (Special Issue), 1998, January, **391**, 309; (*f*) R. P. Rother and S. P. Squinto, *Cell*, 1996, **36**, 185.
6 (*a*) D. K. C. Cooper, *Clin. Trans.*, 1992, **6**, 178; (*b*) U. Galili, *Immunol. Today*, 1993, **14**, 480.
7 W. Zhang, J.-Q. Wang, J. Li, L.-B. Yu and P. G. Wang, *J. Carbohydr. Chem.*, 1999, **18**, 1009.
8 (*a*) J. Wang, X. Chen, W. Zhang, S. Zacharek, Y. Chen and P. G. Wang, *J. Am. Chem. Soc.*, 1999, **121**, 8174; (*b*) J. Fang, X. Chen, W. Zhang, J. Wang, P. R. Andreana and P. G. Wang, *J. Org. Chem.*, 1999, **64**, 4089; (*c*) J. Fang, J. Li, X. Chen, Y. Zhang, J. Wang, Z. Guo, W. Zhang, L. Yu, K. Brew and P. G. Wang, *J. Am. Chem. Soc.*, 1998, **120**, 6635; (*d*) G. Vic, C. H. Tran, M. Scigelova and H. G. Crout, *Chem. Commun.*, 1997, 169.
9 X. Chen, Z.-Y. Liu, J.-Q. Wang, J. Fang, H. Fan and P. G. Wang, *J. Biol. Chem.*, 2000, **275**, 31594.
10 (*a*) S. P. Rannard and N. J. Davis, *Org. Lett.*, 1999, **1**, 933; (*b*) F. M. Houlihan, F. Bouchard and J. M. J. Fréchet, *Macromolecules*, 1986, **19**, 13; (*c*) H. A. Staab, *Angew. Chem., Int. Ed. Engl.*, 1962, **1**, 351.
11 J. Li, S. Zacharek, X. Chen, J.-Q. Wang, W. Zhang, A. Janczuk and P. G. Wang, *Bioorg. Med. Chem.*, 1999, **7**, 1549.
12 G. D. Diana, D. L. Volkots, T. J. Nitz, T. R. Bailey, M. A. Long, N. Vescio, S. Aldous, D. C. Pevear and F. J. Dutko, *J. Med. Chem.*, 1994, **37**, 2421.
13 (*a*) U. Galili, D. C. LaTemple and M. Z. Radic, *Transplantation*, 1998, **65**, 1129; (*b*) U. Galili, M. Ron and R. Sharon, *J. Immunol. Methods*, 1992, **151**, 117.