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Design and synthesis of αGal-conjugated peptide T20 as novel antiviral agent for HIV-immunotargeting

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An efficient chemo-enzymatic synthesis of αGal-conjugated peptide T20 as novel HIV-immuno-targeting agent is described. The synthesis involves chemo-enzymatic preparation of maleimide-functionalized αGal epitope and its chemoselective ligation with the peptide T20. The title compound contains two functional domains: the trisaccharide αGal epitope that binds to human natural anti-Gal antibodies and the 36-amino acid gp41 peptide (T20) that recognizes the gp41 N-terminal ectodomain of the HIV envelope. Biological assays demonstrated that the synthetic conjugate could readily bind to natural anti-Gal antibodies (both IgG and IgM type) in normal human serum and exhibited potent anti-HIV activity even in the absence of human antibodies and complement system. The experimental data suggest that the synthetic αGal-T20 might be valuable for *in vivo* HIV-immuno-targeting *via* antibody-mediated cytotoxicity and/or antibody-dependent, complement-mediated lysis of HIV particles and HIV-infected cells, thus providing an additional dimension of HIV intervention.

Introduction

The human immunodeficiency virus (HIV) is the cause of AIDS.[1–3] The worldwide pandemic of HIV/AIDS urges development of more effective anti-HIV therapy.[4] Hitherto, clinically available anti-HIV drugs belong to two classes of anti-retroviral agents: the reverse transcriptase inhibitor or protease inhibitor.[5] The highly active antiretroviral therapy (HAART) that combines two or more reverse transcriptase/protease inhibitors has prolonged the survival of AIDS patients and significantly reduced viral load of HIV infection. However, the toxicity and emergence of drug-resistant HIV strains associated with these anti-HIV drugs have limited their effectiveness. Recently, a novel 36-amino acid gp41 peptide, T20, was approved by the US Food and Drug Administration (FDA) as a new class of anti-HIV drug. Peptide T20 is a potent HIV-cell fusion inhibitor that exerts its anti-HIV activity through binding to the fusion-essential *N*-terminal region of HIV envelope glycoprotein gp41.[6,7]

To explore new approaches for anti-HIV therapy, we initiated a project aimed at combining the power of antiviral agents and intrinsic human natural antibodies to block HIV infection. It is known that natural anti-Gal antibodies are abundant in humans, which consist of 1–2% total IgG and 3–8% total IgM in human serum.[8] The interaction between human natural anti-Gal antibodies and the αGal epitopes (oligosaccharides with terminal Galα1–3Gal structure) on cells of most mammals such as pigs is mainly responsible for the hyperacute rejection in xenotransplantation.[9–11] Major αGal epitopes that are abundantly expressed on cells of most mammals, except humans, apes, and other Old World monkeys, are Galα1-3Galβ1-4-Glcβ1-R, Galα1-3Galβ1-4GlcNAcβ1-R, and Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-R. We hypothesize that tagging HIV surfaces with αGal epitopes will lead to antibody-mediated cytotoxicity and/or antibody-dependent, complement-mediated lysis of HIV particles and HIV-infected cells. To test the hypothesis, we have designed and synthesized novel glycoconjugates that contain two functional domains, the αGal epitope that binds to human natural antibodies and the HIV-recognizing domain that binds to the HIV envelope. We reasoned that the bi-functional glycoconjugates as mediators will be able to direct human anti-Gal antibodies to HIV and lead to subsequent complement cascade immuno-reactions (Fig. 1). In this paper, we report the design and synthesis of the

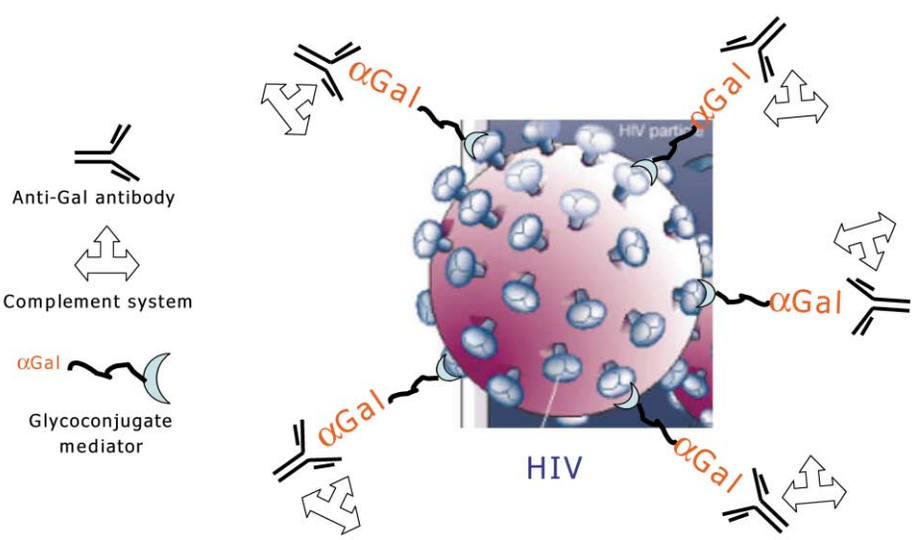

Fig. 1 Schematic description of αGal-mediated antibody coating of HIV surface.

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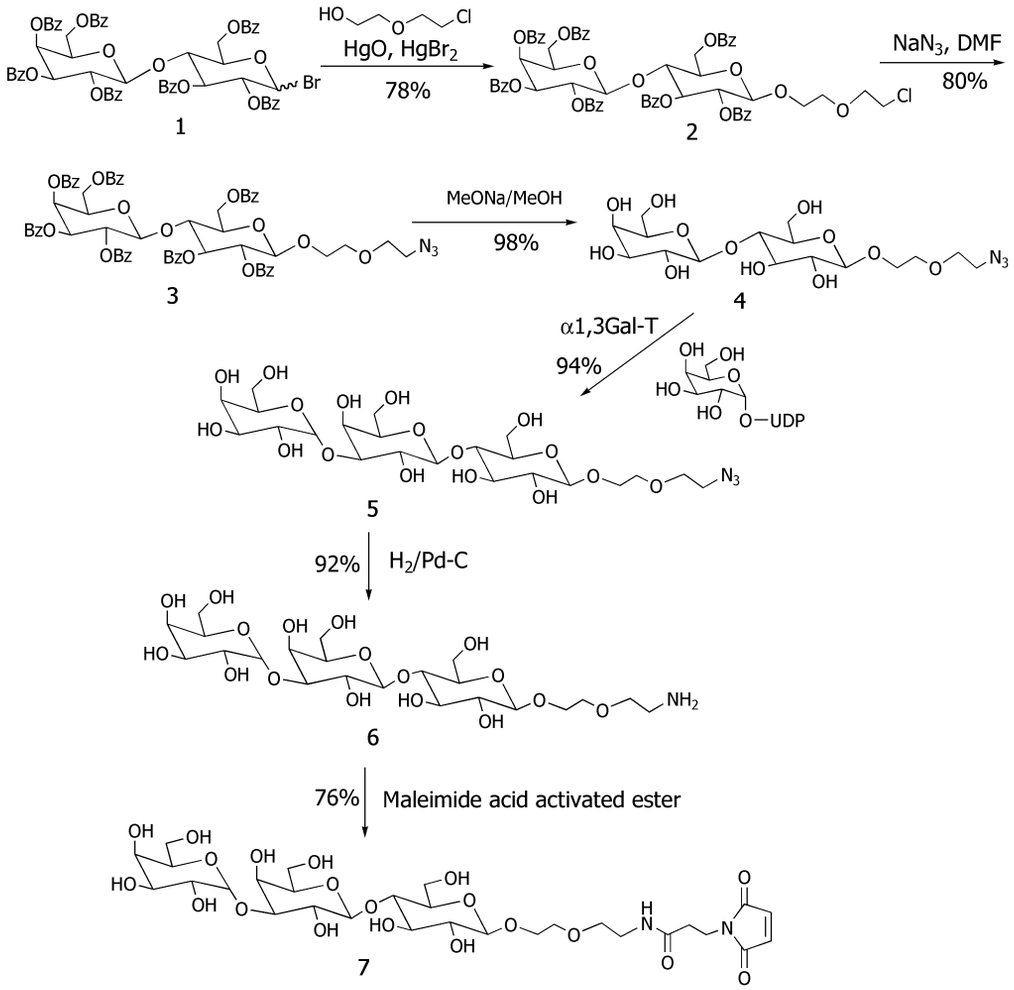


Scheme 1 Synthesis of maleimide-functionalized aGal trisaccharide.

αGal-peptide T20 conjugate. The anti-HIV activity of the synthetic conjugate and its binding to anti-Gal antibodies in human serum are also described.

Results and discussion

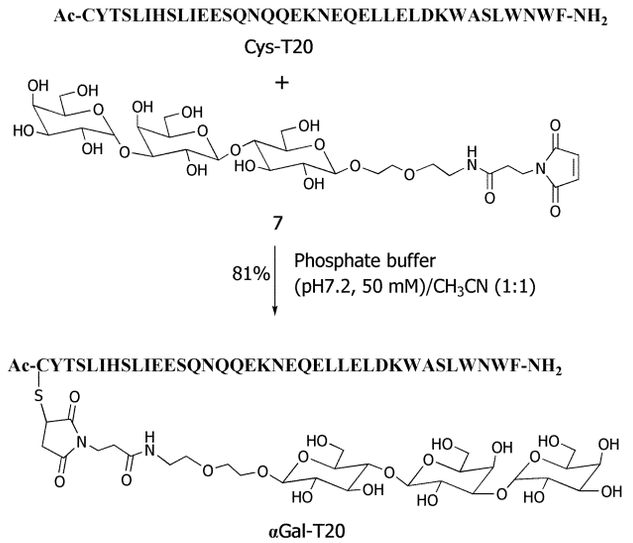
Synthesis of maleimide-functionalized αGal epitope

For the conjugation of the αGal epitope to peptide or other HIV-binding agents, a functionalized αGal epitope that would allow chemo-selective ligation in a later stage would be desirable. Previously, we have applied the maleimide–thiol reaction as a highly chemoselective ligation method for constructing large and complex bio-conjugates in aqueous solution.[12–14] Here we adopted the same strategy for synthesizing the title oligosaccharide–peptide conjugate *via* a maleimide-functionalized αGal epitope. Several chemical and chemoenzymatic methods have already been reported for the synthesis of αGal epitope oligosaccharides and some derivatives.[15–22] Wang *et al.* carried out pioneering work in the field and synthesized various αGal derivatives for biological studies.[23,24] Trisaccharide Galα1-3Galβ1-4Glcβ1-R is one of the major natural αGal epitopes. We used a chemo-enzymatic approach to synthesize the maleimide-functionalized trisaccharide using a recombinant α1-3-galactosyltransferase as the enzyme.[18] The synthesis is summarized in Scheme 1. To install a maleimide functionality in the αGal epitope through a hydrophilic linker, per-*O*-benzoyl lactosyl bromide (**1**)[25] was reacted with 2-(2-chloroethoxy)ethanol under the catalysis of $HgO/HgBr_2$ to give the β-lactoside derivative **2** in 78% yield. The desired β-anomeric configuration was confirmed by the relatively large coupling constant of the anomeric proton ($J_{1,2}$ = 7.8 Hz). Treatment of **2** with NaN_3 in DMF readily converted the chloride into the azide **3** (80%). De-*O*-benzoylation of **3** with MeONa–MeOH gave the free lactoside **4** in quantitative yield. Enzymatic transfer of a galactose moiety from uridine 5′-diphosphate galactose (UDP-Gal) to the 3′-position of the lactoside **4** under the catalysis of the recombinant α1-3-galactosyltransferase afforded the desired trisaccharide derivative **5** in 94% yield. The α-configuration of the newly formed glycosidic linkage was confirmed by the relatively small coupling constant of the H-1″ anomeric proton ($J_{1'',2''}$ = 3.6 Hz). The azido group in **5** was then reduced through catalytic hydrogenation to give the amino compound **6**. Finally, reaction of amine **6** with *N*-[β-maleimidopropyloxy] succinimide ester in an aqueous buffer gave the maleimide-functionalized αGal trisaccharide **7** in 76% yield, which was purified by gel filtration and HPLC and characterized by ESI-MS.

Synthesis of the αGal-conjugated T20

T20 is a potent HIV entry inhibitor consisting of a 36-amino acid sequence derived from the *C*-terminal ectodomain of HIV-1 gp41.[6,7] We synthesized cysteine (Cys)-tagged T20 with the cysteine residue being attached at either the *N*- or *C*-terminus of the 36-amino acid peptide. Our cell fusion experiments indicated that while the *N*-terminal tagged T20 (Cys-T20) maintained the same activity as T20 in inhibiting HIV-cell fusion, the activity of *C*-terminal modified peptide (T20-Cys) showed significantly reduced inhibitory activity (data not shown). Accordingly, we have chosen the *N*-terminus of T20 as the site for selective modification with αGal epitopes. The chemoselective ligation of peptide Cys-T20 and the maleimide-containing trisaccharide **7** proceeded efficiently in a mixed solvent (MeCN–phosphate buffer, pH 7.2) to give the

saccharide–peptide conjugate αGal-T20 (Scheme 2). The product was purified by HPLC and its identity was characterized by ESI-MS (Fig. 2).



Scheme 2 Synthesis of aGal-conjugated peptide T20.

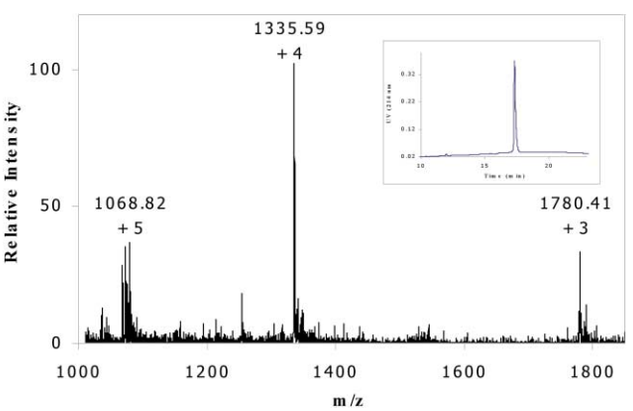


Fig. 2 The HPLC and ESI-MS profiles of synthetic αGal-T20.

Anti-HIV activity and antibody-binding ability of αGal-T20

The synthetic conjugate αGal-T20 was tested for its anti-HIV activity in cell cultures. Peripheral blood mononuclear cells (PBMCs) were used as host cells and HIV-1_{IIIB} strain was used as the virus for infection. Inhibition of viral infection was assessed by measuring the viral reverse transcriptase (RT) activity. As demonstrated in Fig. 3, the synthetic αGal-T20 showed almost the same inhibitory activity against HIV infection as T20 in the absence of human antibodies. The measured IC_{50} (concentration for 50% inhibition) were 8 and 6 nM for αGal-T20 and T20, respectively. The results suggest that attachment of the trisaccharide epitope at the *N*-terminus of T20 does not change its ability to bind to the gp41 region of the HIV envelope.

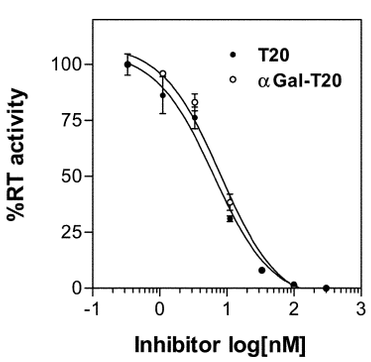


Fig. 3 Anti-HIV activity. Data from the average of two independent experiments were presented. Symbols:●, T20; ○, αGal-T20.

To evaluate whether the synthetic αGal-T20 is able to recognize anti-Gal antibodies in human serum, we performed a simple enzyme-linked immunosorbent assay (ELISA). Mouse laminin, a natural glycoprotein containing multiple αGal epitopes, was included as a positive control in ELISA. αGal-T20 or laminin was immobilized on a microplate and titrated against serial dilutions of normal human serum (male, type AB) that contains natural anti-Gal antibodies. Bound anti-αGal IgG was detected by horseradish peroxidase (HRP) conjugated anti-human IgG antibodies with subsequent color reaction. It was observed that, similar to natural αGal-containing glycoprotein laminin, the synthetic αGal-T20 readily recognized anti-αGal IgG in human serum (Fig. 4). Similar results were observed for anti-αGal IgM in human serum when the bound antibody was detected using HRP conjugated anti-human IgM antibody in ELISA (data not shown). Although it is difficult to provide a quantitative comparison of αGal-T20 and laminin, the data clearly indicate that the synthetic conjugate recognizes both anti-αGal IgG and IgM antibodies in normal human serum. Taken together with the *in vitro* anti-HIV activity of αGal-T20, our data suggest that the synthetic αGal-conjugated T20 may act as a novel bi-functional antiviral agent for synergistic HIV-inhibition and antibody-mediated immuno-targeting of HIV *in vivo*. Therefore, the logical next step of the research is to test the αGal-T20 and additional synthetic αGal-conjugates in animal models to evaluate their *in vivo* anti-HIV activities.

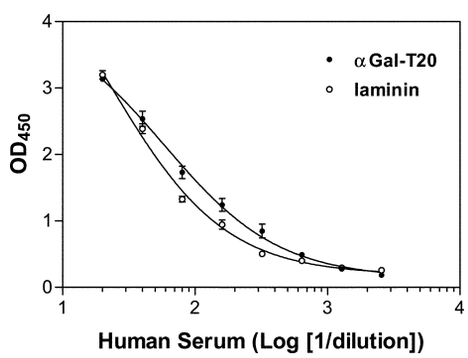


Fig. 4 Anti-Gal antibody binding to immobilized αGal epitopes. Data from the average of two independent experiments were presented. Symbols: ●, αGal-T20, ○, laminin.

Conclusion

We have developed an efficient chemo-enzymatic synthesis of αGal-conjugated peptide T20. The synthetic approach described should be readily applicable for the synthesis of other designed αGal-conjugates. Our preliminary studies indicate that the synthetic αGal-T20 recognizes natural anti-αGal antibodies in normal human serum and meanwhile exhibits potent anti-HIV activity even in the absence of antibodies and complementary systems. Our *in vitro* experimental data suggest that the synthetic αGal-T20 would be valuable for *in vivo* immuno-targeting against HIV *via* antibody-mediated cytotoxicity and/or antibody-dependent, complement-mediated lysis of HIV particles and HIV-infected cells, thus providing an additional dimension of HIV intervention.

Experimental

General

^{1}H NMR spectra were recorded on a Varian 500 with Me_4Si (δ 0) as the internal standard. ESI-MS spectra were measured on a Waters ZMD mass spectrometer. Analytical TLC was performed on glass plates coated with silica gel 60 F254

(E. Merck). Carbohydrates were detected by charring with 10% sulfuric acid in ethanol, and amine was detected by ninhydrin spraying (0.3% in ethanol and acetic acid (97 : 3)). Flash column chromatography was performed with silica gel 60 (EM Science, 230–400 mesh). Analytical HPLC was carried out with a Waters 626 HPLC instrument on a Waters Nova-Pak C18 column (3.9 × 150 mm) at 40 °C. The column was eluted with a suitable gradient of MeCN–H_2O containing 0.1% TFA at a flow rate of 1 mL min^{-1}. Preparative HPLC was performed with a Waters 600 HPLC instrument of a Waters C18 column (Symmetry 300, 19 × 300 mm). The column was eluted with a suitable gradient of MeCN–H_2O containing 0.1% TFA at a flow rate of 12 mL min^{-1}. Mouse laminin, human serum (male, type AB), peroxidase-conjugated goat anti-human IgG and IgM, and TMB were purchased from Sigma. Recombinant α1-3-galactosyltransferase was a gift from Dr Peng George Wang. All other reagents and solvents were purchased from Aldrich/Sigma.

2-(2-Chloroethoxy)ethyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1⟶4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (2). A mixture of hepta-*O*-benzoyl-α-D-lactosyl bromide[25] (1.93 g, 1.7 mmol) and 2-(2-chloroethoxy)ethanol (1.1 g, 8.5 mmol) in dry $CHCl_3$ (20 mL) containing powdered molecular sieves (MS 4 Å, 3 g) were stirred at rt for 30 min. To the suspension were added HgO (600 mg, 2.8 mmol) and HgBr2 (cat.) and the resulting mixture was stirred in the dark at rt for 50 h. After filtration through a pad of Celite, the filtrate was washed with saturated $NaHCO_3$ and water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was subject to silica gel column chromatography with hexane–EtOAc (3 : 2, v/v) as the eluent to afford **2** (1.5 g, 75%): ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.1–7.1 (m, 35H), 5.83 (t, J = 9.15 Hz, 1H, H-3), 5.73–5.77 (m, 2H, H-2′, H-4′), 5.49 (dd, J = 9.52, 11.58 Hz, 1H, H-2), 5.41 (dd, J = 10.25 Hz, 1H, H-3′), 4.92 (d, $J_{1',2'}$ = 8.05 Hz, 1H, H-1′), 4.83 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1), 4.56–4.45 (m, 2H, H-6a,b), 4.29 (t, J = 9.51 Hz, 1H, H-4), 3.96–3.83 (m, 4H, H-5,5′, 6′a, b), 3.78–3.53 (m, 6H), 3.49 (t, 3H); ESI-MS: 1177.80 $(M + H)^+$, 1199.80 $(M + Na)^+$, 1053.71 $(M - O(CH_2)_2–O(CH_2)_2Cl + H)^+$.

2-(2-Azidoethoxy)ethyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1⟶4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (3). To a solution of chloride **2** (1.5 g, 1.45 mmol) in DMF (35 mL) was added NaN_3 (250 mg, 3.8 mmol). The resulting mixture was stirred at 80 °C for 7 h. The solvent was removed under reduced pressure. The residue was dissolved in $CHCl_3$ (60 mL) and washed with brine and water. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was subject to silica gel chromatography using hexane–EtOAc (3 : 2, v/v) as the eluent to give azide **3** (1.37 g, 80%): ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.1–7.1 (m, 35H), 5.82 (t, J = 9.15 Hz, 1H, H-3), 5.74–5.71 (m, 2H, H-2′, H-4′), 5.47 (dd, J = 9.51, 8.78, Hz, 1H, H-2), 5.39 (dd, J = 10.62, 10.25 Hz, 1H, H-3′), 4.90 (d, $J_{1',2'}$ = 7.68 Hz, 1H, H-1′), 4.80 (d, $J_{1,2}$ = 8.05 Hz, 1H, H-1), 4.65–4.45 (m, 2H, H-6a,b), 4.26 (t, J = 9.51 Hz, 1H, H-4), 3.96–3.82 (m, 4H, H-5,5′, 6′a, b), 3.78–3.53 (m, 6H), 3.41 (t, 3H); ESI-MS: 1184.42 $(M + H)^+$, 1206.46 $(M + Na)^+$, 1053.39 $(M - O(CH_2)_2–O(CH_2)_2N_3 + H)^+$.

2-(2-Azidoethoxy)ethyl-β-D-galactopyranosyl-(1⟶4)-β-D-glucopyranoside (4). To a solution of *O*-benzoylated lactoside **3** (60 mg, 51 mmol) in dry MeOH (10 mL) was added a catalytic amount of MeONa in MeOH. The solution was stirred at rt overnight and then neutralized with cation-exchange resin (Dowex 50WX8, H+ form). After filtration, the filtrate was concentrated to dryness under reduced pressure. The residue was crystallized from MeOH–EtOAc to give compound **4** as white crystals (23 mg, 98%): mp 142.5–143.5 °C; selected ^{1}H NMR data (500 MHz, D_2O): δ 4.55 (d, J = 7.68 Hz, 1H, H-1), 4.47 (d, J = 8.05 Hz, 1H, H-1′), 3.55–4.11 (m, 17H), 3.54 (t, 2H, CH_2N_3), 3.36 (t, 1H); ESI-MS: 456.36 $(M + H)^+$, 478.31 $(M + Na)^+$.

2-(2-Azidoethoxy)ethyl α-D-galactopyranosyl-(1⟶3)-β-D-galactopyranosyl-(1⟶4)-β-D-glucopyranoside (5). A solution of compound **4** (11 mg, 24.2 μmol) and UDP-Gal (20 mg, 32.7 μmol) in a Tris-HCl buffer (2 mL, 50 mM, pH 7.0) containing bovine serum albumin (BSA) (0.1%), $MnCl_2$ (3.75 mg, 5.1 mmol), and recombinant α1-3-galactosyltransferase (0.4 unit) was gently shaken at rt After 48 h, TLC (2-propanol–EtOAc–water, 2 : 2 : 1, v/v) showed the disappearance of the disaccharide acceptor and the formation of a slower moving product. The mixture was passed through a column of anion-exchanging resin (Dowex-1, chloride form). The eluate was concentrated and loaded onto a column of Sephadex G-10 using water as the eluent. The fractions containing the trisaccharide product were collected and lyophilized to give the trisaccharide **5** (14 mg, 94%): selected ^{1}H NMR data (500 MHz, D_2O): δ 5.1 (d, J = 3.6 Hz, 1H), 4.48 (d, J = 7.32 Hz, 1H, H-1), 4.46 (d, J = 7.32 Hz, 1H, H-1′), 4.14 (m, 2H), 3.50–4.04 (m, 21H), 3.47 (t, 2H, CH_2N_3), 3.30 (t, 1H); ESI-MS: 640.30 $(M + Na)^+$, 618.29 $(M + H)^+$, 489.06 $(M - O(CH_2)_2-O(CH_2)_2N_3 + H)^+$.

2-(2-Aminoethoxy)-ethyl α-D-galactopyranosyl-(1⟶3)-β-D-galactopyranosyl-(1⟶4)-β-D-glucopyranoside (6). A solution of azide **5** (12 mg, 20.28 μmol) in MeOH (10 mL) containing Pd/C (5%, 3 mg) was stirred under an H_2 atmosphere at rt overnight. The solution was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was dissolved in water and lyophilized to give the amine **6** (11 mg, 92%). Selected ^{1}H NMR data (500 MHz, D_2O): δ 5.18 (d, J = 3.6 Hz, 1H), 4.56 (d, J = 6.58 Hz, 1H, H-1), 4.55 (d, J = 7.69 Hz, 1H, H-1′), 4.22 (m, 2H), 3.56–4.12 (m, 24H), 3.38 (t, 1H); ESI-MS: 592.53 $(M + H)^+$.

2-(2-β-Maleimidopropylamidoethoxy)ethyl α-D-galactopyranosyl-(1⟶3)-β-D-galactopyranosyl-(1⟶4)-β-D-glucopyranoside (7). To a solution of amine **6** (32 mg, 61.6 μmol) in a phosphate buffer (5 mL, 50 mM, pH 7.2) was added a solution of *N*-(β-maleimidopropyloxy)succinimide ester (25 mg, 96 μmol) in MeCN (3 mL). The mixture was stirred at rt for 3 h when TLC showed the disapperance of the amine **6**. The reaction mixture was lyophilized and the product was then purified by HPLC (0–30% MeCN) to give compound **7** (35 mg, 76%). Selected ^{1}H NMR data (500 MHz, D_2O): δ 6.76 (s, 2H), 5.08 (d, J = 3.6 Hz, 1H), 4.46 (d, J = 8.50 Hz, 1H, H-1), 4.45 (d, J = 8.50 Hz, 1H, H-1′), 4.13 (m, 2H), 3.58–3.98 (m, 24H), 2.54 (t, 2H), 2.39 (t, 2H); ESI-MS: 743.44 $(M + H)^+$, 765.41 $(M + Na)^+$.

Chemoselective ligation of peptide Cys-T20 and maleimide 7. Peptide Cys-T20 was synthesized on a Pioneer solid phase peptide synthesizer using Fmoc chemistry and was purified by HPLC according to our previously described procedure.[12,14] A mixture of Cys-T20 (9 mg, 1.95 μmol) and maleimide **7** (1.45 mg, 1.95 μmol) in a mixed solvent of phosphate buffer (pH 7.2, 50 mM, and 2.5 mL) and MeCN (1.5 mL) was gently shaken at rt for 3 h. The reaction mixture was then lyophilized and the ligation product was purified by preparative HPLC to give αGal-T20 (8.4 mg, 81%). Analytical HPLC, t_R = 17.3 min (0–70% MeCN containing 0.1% TFA in 20 min); ESI-MS 1780.41 $(M + 3H)^{3+}$, 1335.59 $(M + 4H)^{4+}$, 1068.82 $(M + 5H)^{5+}$.

Anti-viral assays. Peripheral blood mononuclear cells (PBMCs) were separated from whole blood of HIV-seronegative donors by density centrifugation over Ficoll-Hypaque (Sigma). The culture medium consisted of RPMI

supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine and penicillin/streptomycin (Gibco, Grand Island, NY). For infection studies PBMCs were stimulated with 2.5 μg ml^{-1} phytohemagglutinin (PHA; Boehringer Mannheim, Indianapolis, IN) for 3 days. Stimulated PBMCs were infected by incubation with HIV-1_{IIIB} at a multiplicity of infection of 1000 $TCID_{50}/10^6$ PBMC for 2 h. PBMCs were then washed three times with PBS and cultured at 37 °C in RPMI/10% FBS supplemented with 100 units ml^{-1} rIL-2 (Boehringer Mannheim) and the antiviral agents (αGal-T20 or T20). PBMCs were seeded in 96-well flat-bottom plates at a density of 2×10^5 PBMCs per 200 μl. Following 3 days of culture, half of the medium was replaced with fresh medium containing IL-2 and the antiviral agents (αGal-T20 or T20). After 7 days of culture, virus production in the culture supernatant was assayed by measuring HIV-1 reverse transcriptase activity as described.[26] Cell viability in culture in the presence or absence of antiviral agents was measured by commercial MTT assay, as per the manufacturer's instructions (Boehringer Mannheim).

Binding of αGal-T20 to human anti-Gal antibodies. Microtiter ELISA plates were coated with αGal-T20 (10 μg mL^{-1}) or mouse laminin (10 μg mL^{-1}) at 4 °C overnight. After washings with PBS/0.5% Tween-20, non-specific binding was blocked with 5% BSA in PBS at rt for 1 h. The plates were then washed three times with PBS/0.5% Tween-20. Serial dilutions (1 : 2) of normal human serum (male, type AB) samples were applied to the plates and incubated at 37 °C for 1 h, then washed with PBS/0.5% Tween-20. To the plates was added a solution of 1 : 3000 diluted horseradish peroxidase (HRP)-conjugated goat anti-human IgG or anti-human IgM in 0.5%BSA/PBS. After incubation for 1 h at 37 °C, the plates were washed again and a solution of 3,3′,5,5′-tetramethyl benzidine (TMB) was added. Color was allowed to develop for 5 min, and the reaction was then quenched by adding a solution of 0.5 M H_2SO_4 to each well. The optical density was then measured at 450 nm.

Acknowledgements

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References

1 R. C. Gallo, *Science*, 2002, **298**, 1728–30.
2 L. Montagnier, *Science*, 2002, **298**, 1727–8.
3 R. C. Gallo and L. Montagnier, *Science*, 2002, **298**, 1730–1.
4 UNAIDS, "AIDS epidemic update: December 2002," World Health Organization, 2002.
5 D. D. Richman, *Nature*, 2001, **410**, 995–1001.
6 J. M. Kilby, S. Hopkins, T. M. Venetta, B. DiMassimo, G. A. Cloud, J. Y. Lee, L. Alldredge, E. Hunter, D. Lambert, D. Bolognesi, T. Matthews, M. R. Johnson, M. A. Nowak, G. M. Shaw and M. S. Saag, *Nat. Med.*, 1998, **4**, 1302–7.
7 C. T. Wild, D. C. Shugars, T. K. Greenwell, C. B. McDanal and T. J. Matthews, *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 9770–4.
8 R. P. Rother and S. P. Squinto, *Cell*, 1996, **86**, 185–8.
9 I. P. Alwayn, M. Basker, L. Buhler and D. K. Cooper, *Xenotransplantation*, 1999, **6**, 157–68.
10 M. S. Sandrin, H. A. Vaughan, P. L. Dabkowski and I. F. McKenzie, *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 11391–5.
11 U. Galili, *Immunol. Today*, 1993, **14**, 480–2.
12 H. Li and L. X. Wang, *Org. Biomol. Chem.*, 2003, **1**, 3507–13.
13 J. Ni, S. Singh and L. X. Wang, *Bioconjug. Chem.*, 2003, **14**, 232–8.
14 L. X. Wang, J. Ni and S. Singh, *Bioorg. Med. Chem.*, 2003, **11**, 129–36.
15 C. H. Hokke, A. Zervosen, L. Elling, D. H. Joziasse and D. H. van den Eijnden, *Glycoconjugate J.*, 1996, **13**, 687–92.
16 K. G. I. Nilsson, *Tetrahedron Lett.*, 1997, **38**, 133–36.
17 G. Vic, C. H. Tran, M. Scigelova and D. H. G. Crout, *Chem. Commun.*, 1997, 169–70.
18 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**, 6635–38.
19 J. Li, S. Zacharek, X. Chen, J. Wang, W. Zhang, A. Janczuk and P. G. Wang, *Bioorg. Med. Chem.*, 1999, **7**, 1549–58.
20 S. Hanessian, H. K. Huynh, G. V. Reddy, R. O. Duthaler, A. Katopodis, M. B. Streiff, W. Kinzy and R. Oehrlein, *Tetrahedron*, 2001, **57**, 3281–90.
21 Y. Chen, W. Zhang, X. Chen, J. Wang and P. G. Wang, *J. Chem. Soc., Perkin Trans. 1*, 2001, 1716–22.
22 D. Ramos, P. Rollin and W. Klaffke, *J. Org. Chem.*, 2001, **66**, 2948–56.
23 J. Wang, X. Chen, W. Zhang, S. Zacharek, Y. Chen and P. G. Wang, *J. Am. Chem. Soc.*, 1999, **121**, 8174–81.
24 X. Chen, P. R. Andreana and P. G. Wang, *Curr. Opin. Chem. Biol.*, 1999, **3**, 650–58.
25 F. W. Lichtenthaler, E. Kaji and S. Weprek, *J. Org. Chem.*, 1985, **50**, 3505–15.
26 R. L. Willey, D. H. Smith, L. A. Lasky, T. S. Theodore, P. L. Earl, B. Moss, D. J. Capon and M. A. Martin, *J. Virol.*, 1988, **62**, 139–47.