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Synthesis of 3-*O*-sulfonated heparan sulfate octasaccharides that inhibit the herpes simplex virus type 1 host–cell interaction

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Cell surface carbohydrates play significant roles in a number of biologically important processes. Heparan sulfate, for instance, is a ubiquitously distributed polysulfated polysaccharide that is involved, among other things, in the initial step of herpes simplex virus type 1 (HSV-1) infection. The virus interacts with cell-surface heparan sulfate to facilitate host-cell attachment and entry. 3-*O*-Sulfonated heparan sulfate has been found to function as an HSV-1 entry receptor. Achieving a complete understanding of these interactions requires the chemical synthesis of such oligosaccharides, but this remains challenging. Here, we present a convenient approach for the synthesis of two irregular 3-*O*-sulfonated heparan sulfate octasaccharides, making use of a key disaccharide intermediate to acquire different building blocks for the oligosaccharide chain assembly. Despite substantial structural differences, the prepared 3-*O*-sulfonated sugars blocked viral infection in a dosage-dependent manner with remarkable similarity to one another.

Heparan sulfate (HS) is a linear polysaccharide that is widespread on the cell surface and in the extracellular matrix, as well as in the basement membrane[1]. Commonly found as a component of proteoglycans, this sugar is polysulfated and consists of alternating 1 → 4-linked uronic acid (either β-D-glucuronic acid (GlcUA) or α-L-iduronic acid (IdoUA)) and α-D-glucosamine (GlcN) residues[2]. The commercial anticoagulant heparin is a structural relative of HS that is typically sequestered *in vivo* by mastocytes[3]. The biosynthesis of HS and heparin includes the formation of a bridging tetrasaccharide attached to serine side chains of a core protein, followed by the assembly of a long precursor chain characterized by the GlcUA–*N*-acetyl-D-glucosamine (GlcNAc) repeating pattern. Subsequent enzyme-mediated *N*-deacetylation/*N*-sulfonation, uronic acid 5-*C*-epimerization, uronic acid 2-*O*-sulfonation and glucosamine 6-*O*- and 3-*O*-sulfonations generate the mature polysaccharide[4]. The incomplete nature of these modifications and the varying specificities of participating enzyme isoforms deliver a structure with extensive microheterogeneity[5]. Through the variable make-up of its primary sequence, HS is able to mediate and/or modulate the activity of a host of biomolecular agents directly affecting key physiological events, including blood coagulation, tissue repair and remodelling, development, angiogenesis, and bacterial and viral infections[6].

Herpes simplex virus type 1 (HSV-1), a prevalent human pathogen causing chronic and recurrent mucocutaneous lesions, belongs to the neurotropic subgroup of the herpes virus family[7,8]. Cell-surface HS proteoglycans play dual roles not only in assisting cellular attachment of HSV-1, but also in inducing viral entry into the host cell[9,10]. The virion envelope glycoproteins gB and gC bind HS chains, which are richly expressed in filopodia-like projections on the cell surface[11]. HS-attached virions are then transported onto the cell body, where three known viral entry receptor types (herpes virus entry mediator, nectin-1 and a unique 3-*O*-sulfonated HS) are located[12,13]. The receptor interacts with gD (ref. 14), another envelope glycoprotein, altering its conformation and initiating viral penetration through the formation of a fusion-active complex that also includes gB and the gH/gL heterodimer[15,16]. In 2002, the literature reported the first gD-binding 3-*O*-sulfonated octasaccharide generated from a library of partially depolymerized HS by the HS-glucosaminyl-3-*O*-sulfotransferase isoform 3 (3-OST-3)[17]. Represented here by compound **1**, the octasaccharide specially carries the 3-*O*-sulfonate group as well as a free amino group at the reducing end residue (Fig. 1). The fourth residue from the reducing end was not fully established and could be either IdoUA2S or GlcUA2S (2S: 2-*O*-sulfonate). Based on the identified structure, four likely variants could be extrapolated for the gD-binding sequence. Recently, compound **2**, furnished by the 3-OST-3-mediated sulfonation of a heparin-degraded octasaccharide, was identified to inhibit both attachment and entry processes[18]. The specific 3-*O*-sulfonate group was located on the third residue from the reducing end. The less defined non-reducing end residue in this structure offered two possibilities for the natural HS chain sequence.

A complete understanding of the nature of HS–protein interaction necessitates structurally well-defined oligosaccharides accessible mainly through chemical synthesis. Synthetic efforts directed towards the heparin–antithrombin interaction formed the basis of the anticoagulant Fondaparinux, now in clinical use[19]. However, although the chemical synthesis of the regular sequences appearing in HS/heparin, particularly the IdoUA2S–GlcNS6S couple (GlcNS6S: *N*- and 6-*O*-sulfonated GlcN), has been well documented[20–28], the preparation of irregular structures remains a considerable hurdle. In fact, before this article, no report had been made about the synthesis of irregular HS oligosaccharides longer than six residues, and an attempt to construct a gD-binding

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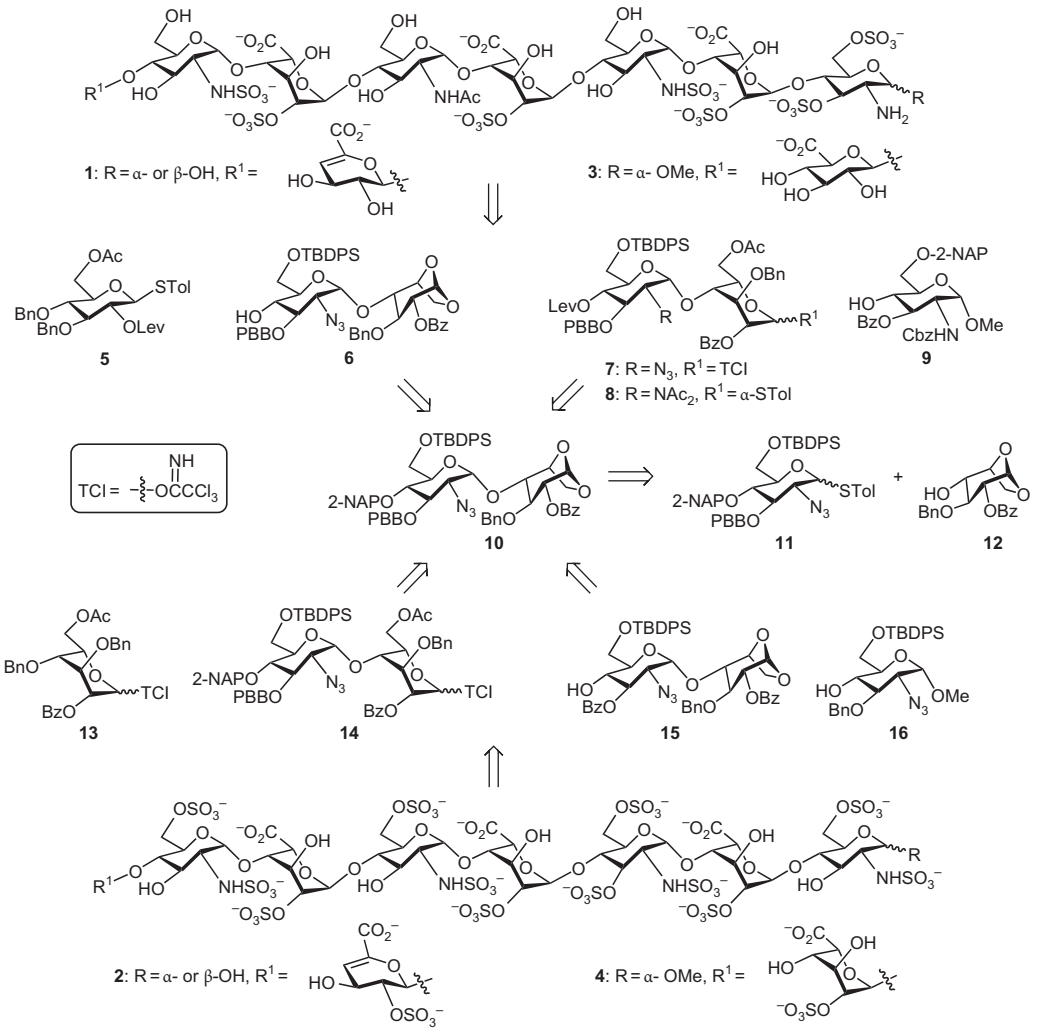

Figure 1 | Specific 3-*O*-sulfonated octasaccharides 1 and 2 and retro-synthetic design of the corresponding α-methylated glycosides 3 and 4. Synthetic targets **3** and **4** were based on the structure of compounds **1** and **2**, respectively. A common disaccharide intermediate **10**, prepared from the D-glucosamine-derived donor **11** and 1,6-anhydro-L-idopyranosyl acceptor **12**, was used in the generation of several disaccharide building blocks for the total synthesis of compounds **3** and **4**. Ac, acetyl; Bn, benzyl; Bz, benzoyl; Cbz, benzyloxycarbonyl; Lev, levulinyl; 2-NAP, 2-naphthylmethyl; PBB, *p*-bromobenzyl; STol, thiotoluenyl; TBDPS, *tert*-butyldiphenylsilyl; TCI, trichloroacetimidate.

octasaccharide based on compound **1** resulted in failure at the hexasaccharide level[29]. In an effort to improve the current knowledge about the interaction between HSV-1 and HS, we present herein a concise synthesis of the irregular α-methylated octasaccharides **3** and **4**, deducible from the recognized 3-*O*-sulfonated structures **1** and **2**, respectively. The ability of these compounds to inhibit HSV-1 infection of Vero cells has also been evaluated.

Results and discussion

Retrosynthesis. Notable concerns posed by the preparation of compounds **3** and **4** are the manner of chain backbone assembly, regio- and stereochemical control in glycosidic bond formation, the different functional make-up of the amino groups (that is, $NHSO_3^-$, NHAc and NH_2), and the differentiation of alcohol groups that would remain free, oxidized or *O*-sulfonated. For octasaccharide **3**, GlcUA was selected as the non-reducing end unit to ease the identification of α/β-stereochemistry, and IdoUA2S was arbitrarily chosen as the fourth residue from the reducing end. Conversely, we decided to use IdoUA2S at the non-reducing end of octasaccharide **4** in view of the semi-repeating pattern of compound **2**. The difficulty inherent in the synthesis of the target compounds compelled us to try several approaches, of which the concept illustrated in Fig. 1 was eventually adopted because of its robustness and simplicity.

We initially encountered some trouble in making the glycosidic bond between the third and fourth residues from the reducing end of octasaccharide **3**. The trifluoroacetyl group used to block the amino group that would eventually become acetylated also presented deprotection problems. After some exploration, a promising route was carried out using disaccharide building blocks **6**–**8** for the core residues and the monosaccharide derivatives **5** and **9** as non-reducing and reducing end units, respectively. The chain would be assembled by the initial couplings of compounds **8**, **7** and **9** to form a pentasaccharide. Here, the levulinyl (Lev) groups would allow the generation of glycosyl acceptors for further condensation with the trichloroacetimidate (TCI) and thiotoluenyl (STol) donors. The octasaccharide would then be accessible by the introduction of a trisaccharide fashioned from compounds **5** and **6**. The 1,2-*trans* stereoselectivity of these glycosidations would be ensured by the adjacent Lev and benzoyl (Bz) groups via neighbouring-group participation. The orthogonal azide (N_3), *N*,*N*-diacetyl (NAc_2) and benzylcarbamate (NHCbz) were designated as masking groups, leading to $NHSO_3^-$, NHAc and NH_2, respectively. Together with Bz, the primary acetyl (Ac) groups could be cleaved under basic

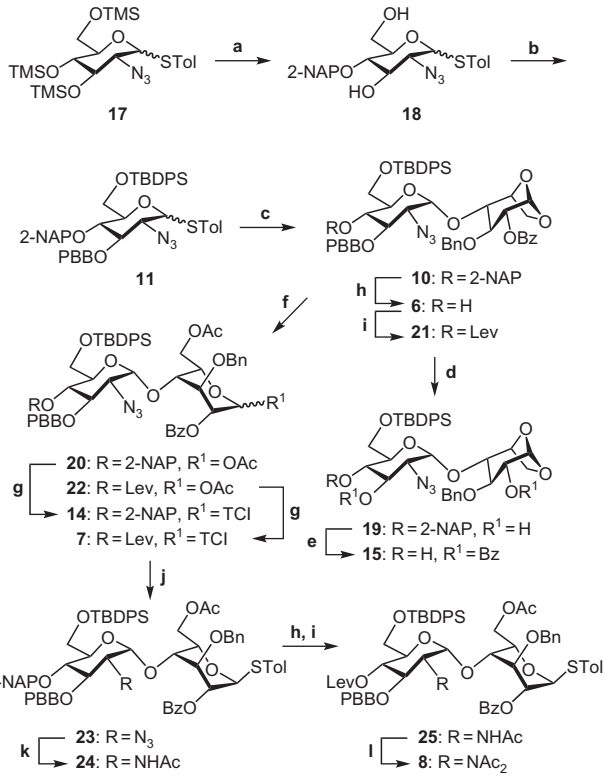

Figure 2 | Preparation of disaccharide building blocks 6, 7, 8, 14 and 15. **a**, 2-$C_{10}H_9CHO$, TMSOTf, CH_2Cl_2, 2 h; BH_3·THF, TMSOTf, 77% (one pot); **b**, TBDPSCl, DMAP, Et_3N, CH_2Cl_2; NaH, *p*-bromobenzyl bromide, DMF, 71% (one pot); **c**, **12**, NIS, TfOH, CH_2Cl_2, −78 °C → −20 °C, 4 h, 79%; **d**, (1) $Pd_2(dba)_3$, *N*-methylaniline, (*o*-biphenyl)P(*t*-Bu)$_2$, sodium *tert*-butoxide, toluene, 80 °C, 5 h; (2) $SnCl_4$, CH_2Cl_2, 10 min, 63% (two steps); **e**, (1) Benzoyl chloride, Et_3N, DMAP, CH_2Cl_2, 89%; (2) DDQ, CH_2Cl_2/H_2O (18/1, v/v), 87%; **f**, $Cu(OTf)_2$, Ac_2O, **20**: 95%, **22**: 94%; **g**, (1) Saturated $NH_{3(g)}$ in MeOH/THF solution; (2) CCl_3CN, K_2CO_3, CH_2Cl_2, **14**: 88% (two steps), **7**: 90% (two steps); **h**, DDQ, CH_2Cl_2/H_2O (18/1, v/v), 4 h, **6**: 93%; **i**, Lev_2O, Et_3N, DMAP, CH_2Cl_2, **21**: 95%, **25**: 81% (two steps); **j**, TMSSTol, ZnI_2, CH_2Cl_2, 3 h, 92%; **k**, Thioacetic acid, Pyr, $CHCl_3$, 87%; **l**, Isopropenyl acetate, *p*-toluenesulfonic acid, 65 °C, 78%. Ac_2O, acetic anhydride; $Cu(OTf)_2$, copper(II) trifluoromethanesulfonate; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; DMAP, 4-(*N*,*N*-dimethylamino)pyridine; DMF, *N*,*N*-dimethylformamide; Lev_2O, levulinic anhydride; NIS, *N*-iodosuccinimide; $Pd_2(dba)_3$, tris(dibenzylideneacetone)dipalladium; Pyr, pyridine; TBDPSCl, *tert*-butylchlorodiphenylsilane; TfOH, trifluoromethanesulfonic acid; THF, tetrahydrofuran; TMS, trimethylsilyl; TMSOTf, trimethylsilyl trifluoromethanesulfonate; TMSSTol, trimethyl(4-methylphenylthio)silane.

conditions and the primary hydroxyls would be regioselectively oxidized to the corresponding carboxylate. The 2-naphthylmethyl (2-NAP) group temporarily protects the sole primary alcohol that would be sulfonated simultaneously with the debenzoylated secondary alcohols. All the ether groups (benzyl (Bn), *p*-bromobenzyl (PBB) and *tert*-butyldiphenylsilyl (TBDPS)) act as permanent protection and would be removed in the late stage of the synthesis to reveal the free hydroxyls.

The envisioned strategy for octasaccharide **4** assembly involved building blocks **13**–**16**. A hexasaccharide, formed by two successive elongations of the disaccharide acceptor **15** with the disaccharide donor **14**, would be capped by the monosaccharide derivatives **13** and **16** at the non-reducing and reducing ends, respectively. Chain elongation would be made possible by the 2-NAP group, enabling transformation to the glycosyl acceptor and the

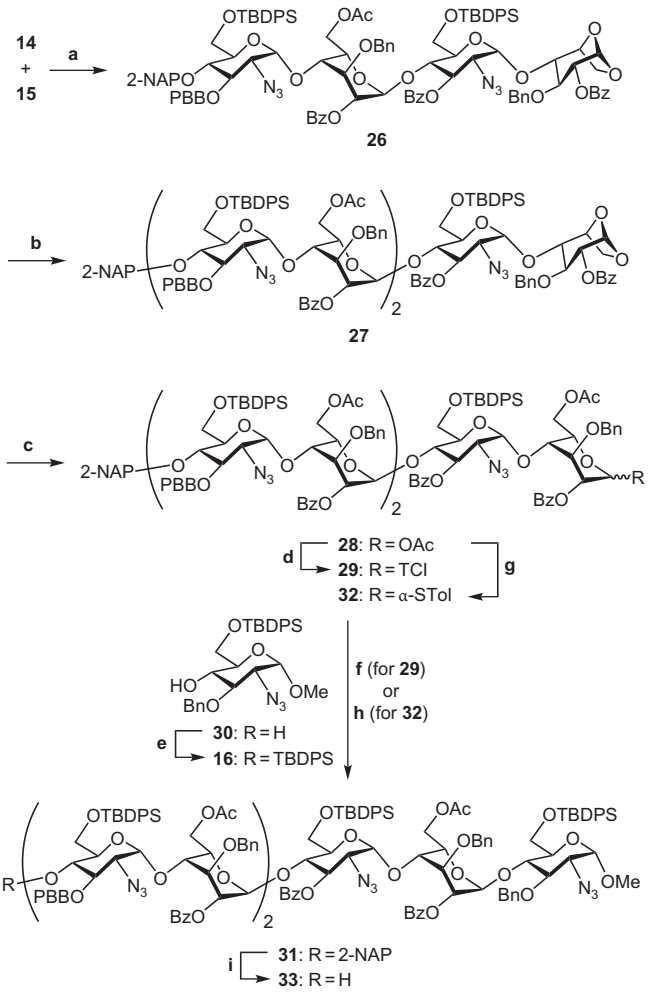

Figure 3 | Preparation of heptasaccharide acceptor 33. **a**, TMSOTf, CH_2Cl_2, −78 °C → −40 °C → 0 °C, 2 h, 83%; **b**, (1) DDQ, CH_2Cl_2/H_2O (18/1, v/v), 2 h, 82%; (2) **14**, TMSOTf, CH_2Cl_2, −78 °C → −40 °C → 0 °C, 2 h, 76%; **c**, $Cu(OTf)_2$, Ac_2O, 83%; **d**, (1) Saturated $NH_{3(g)}$ in MeOH/THF (1/10, v/v), 76%; (2) CCl_3CN, K_2CO_3, CH_2Cl_2, 92%; **e**, TBDPSCl, Et_3N, DMAP, CH_2Cl_2, 3.5 h, 92%; **f**, TMSOTf, CH_2Cl_2, −78 °C → 0 °C, 2 h, 76%; **g**, TMSSTol, ZnI_2, CH_2Cl_2, 2 h, 92%; **h**, NIS, TfOH, CH_2Cl_2, −78 °C → 0 °C, 2 h, 76%; **i**, DDQ, CH_2Cl_2/H_2O (18/1, v/v), 2 h, 74%.

1,6-anhydro ring in **15**, which could allow facile incorporation of a leaving group for further coupling. In addition to the hydroxyl positions protected by the Bz groups, the orthogonal TBDPS moieties also mask the primary alcohols that would ultimately hold sulfonate groups. PBB, Bn, N_3 and Ac groups have roles similar to those stated for compound **3**.

All the disaccharide building blocks **6**, **7**, **8**, **14** and **15** would be accessible through simple transformations from the common intermediate **10**, which could be prepared by coupling monosaccharide units **11** and **12**. Conceptually, the bulky TBDPS group and the non-participating 2-*C*-N_3 group would enhance the stereoselective α-glycosidation of the D-glucosamine-derived donor **11**. Aside from this, the N_3 group could be readily manipulated into various amine derivatives. Both the PBB and 2-NAP groups could be selectively cleaved and transformed into other functional moieties as necessary.

Disaccharide building block syntheses. The preparative routes for the disaccharide derivatives are depicted in Fig. 2. Through our recently conceived regioselective one-pot protection strategy[30–32], the 3,4,6-tri-*O*-trimethylsilylated thioglycoside **17** was transformed into the 3,6-diol **18** by a trimethylsilyl trifluoromethanesulfonate

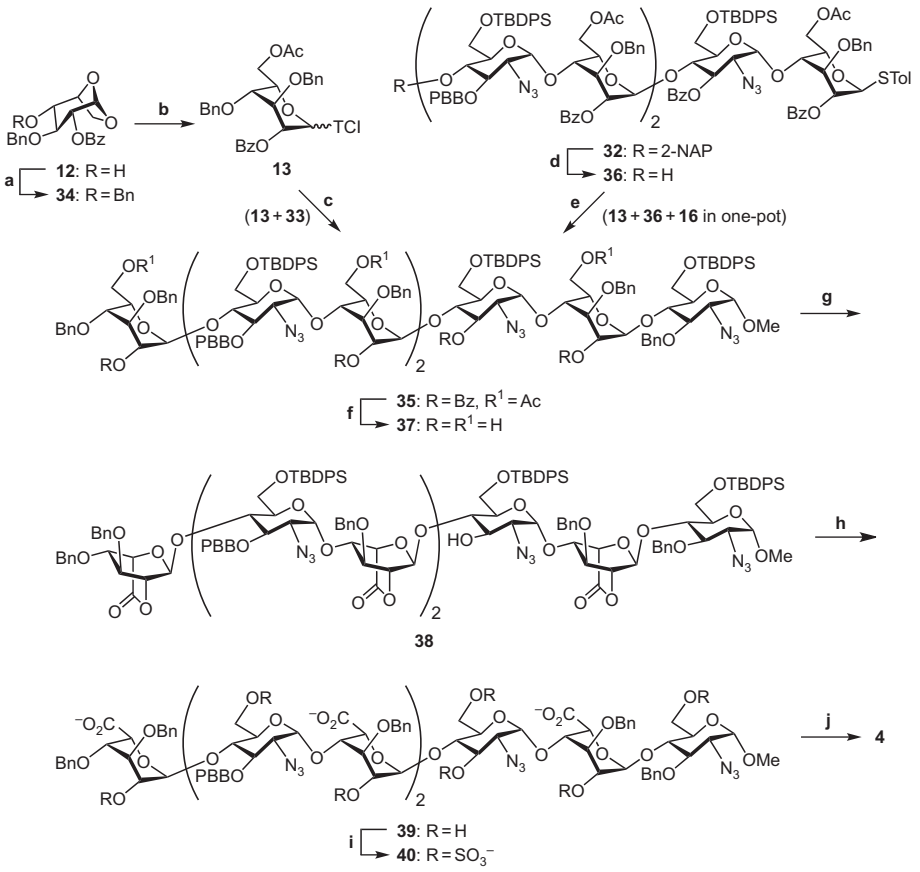

Figure 4 | Synthesis of target octasaccharide 4. a, Ag_2O, BnBr, CH_2Cl_2, 83%; **b**, (1) $Cu(OTf)_2$, Ac_2O, 90%; (2) saturated $NH_{3(g)}$ in MeOH/THF (1/10, v/v), 83%; (3) CCl_3CN, K_2CO_3, CH_2Cl_2, 97%; **c**, **13**, TMSOTf, CH_2Cl_2, −78 °C → −40 °C → 0 °C, 2 h, 78%; **d**, DDQ, CH_2Cl_2/H_2O (18/1, v/v), 2 h, 71%; **e**, **13**, TfOH, CH_2Cl_2, −78 °C → −40 °C → −20 °C, 2 h; **16**, NIS, TfOH, −78 °C → 0 °C, 3 h, 31% (one pot); **f**, NaOMe, MeOH/CH_2Cl_2 (1/1, v/v), 78%; **g**, TEMPO, BAIB, CH_2Cl_2/H_2O (1/1, v/v), 68%; **h**, (1) tetra-*n*-butylammonium fluoride, AcOH, THF; (2) LiOH, THF, 1 h, 67% (two steps); **i**, SO_3·Et_3N, DMF, 60 °C, 62%; **j**, (1) $Pd(OH)_2/C$, H_2, phosphate buffer pH = 7, MeOH; (2) SO_3·Pyr, pH = 9.5, H_2O, 3 h, 72% (two steps). AcOH, acetic acid; BAIB, bis(acetoxy)iodo-benzene; BnBr, benzyl bromide; TEMPO, 2,2,6,6-tetramethyl-1-piperidinyloxyl free radical.

(TMSOTf)-promoted two-step process. Regioselective 6-*O*-silylation of **18** catalysed by 4-(*N*,*N*-dimethylamino)pyridine (DMAP), followed by 3-*O*-*p*-bromobenzylation under Williamson's conditions, furnished the fully protected thioglycoside **11** (71%) in a one-pot manner. Coupling of the glycosyl donor **11** with alcohol **12** (ref. 33) via an *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) promoter combination led to the desired α-linked disaccharide **10** (79%, $J = 3.8$ Hz for 1′-H) as a single isomer. Removal of the PBB group at the 3′-O position of **10** was carried out through palladium-catalysed amination with *N*-methylaniline, followed by treatment with tin tetrachloride[34]. Cleavage of the 2-*O*-Bz group also occurred in this transformation, giving the 2,3′-diol **19** in 63% yield. Dibenzoylation of **19** followed by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)-mediated cleavage of the 4′-*O*-2-NAP group provided the desired alcohol **15**.

1,6-Anhydro ring opening in compound **10** with acetic anhydride (Ac_2O) using copper(II) trifluoromethanesulfonate ($Cu(OTf)_2$) as catalyst[35] afforded the 1,6-diacetate **20** in excellent yield. The 2-NAP and TBDPS moieties were stable under this condition, but the *p*-methoxybenzyl group did not survive when used in place of 2-NAP. Regioselective 1-*O*-deacetylation of **20** with saturated $NH_{3(g)}$ gave the 1-alcohol, which was reacted with CCl_3CN and K_2CO_3 to obtain the glycosyl donor **14**.

The 2-NAP group in compound **10** was removed by DDQ, leaving the 4′-alcohol **6** in 93% yield. Installation of Lev in the free hydroxyl of **6** using levulinic anhydride in the presence of DMAP delivered ester **21**, which underwent acetolysis to furnish the fully protected disaccharide **22**. Treatment of **22** with saturated $NH_{3(g)}$ followed by subsequent imidation provided the corresponding TCI derivative **7**.

Compound **8**, with NAc_2 protection and a STol leaving group, was generated via intermediate **20**. We opted to form the thioglycoside, because NAc_2 would not withstand the anomeric deacetylation required before imidate formation. Thus, conversion of the 1-C acetate of **20** into the thioether was carried out by treatment with trimethyl(4-methylphenylthio)silane (TMSSTol) and ZnI_2, generating the corresponding thioglycoside **23** in excellent 92% yield. This ZnI_2-promoted thioether substitution at the anomeric centre tolerated different functional groups in the substrate, unlike the conventional BF_3·Et_2O/*p*-thiocresol tandem. Subsequently, azido group transformation in **23** using thioacetic acid yielded the *N*-acetylated derivative **24** (87%). Successive 4′-*O*-denaphthylmethylation and levulinylation led to compound **25** (81% in two steps), which, on reaction with isopropenyl acetate in catalytic *p*-toluenesulfonic acid, provided the desired building block **8** in 78% yield.

Synthesis of compound 4. The preparation of the target octasaccharide **4** started from the TMSOTf-promoted coupling of the disaccharide donor **14** with the 4′-alcohol **15** (Fig. 3). The fully protected tetrasaccharide **26**, isolated in 83% yield as a single isomer, was treated with DDQ delivering the corresponding 4‴-alcohol. Further elongation with **14** afforded the desired hexasaccharide **27** (76%). Acetolysis of the 1,6-anhydro ring in **27** furnished the ester **28** (83%), which underwent regioselective 1-*O*-

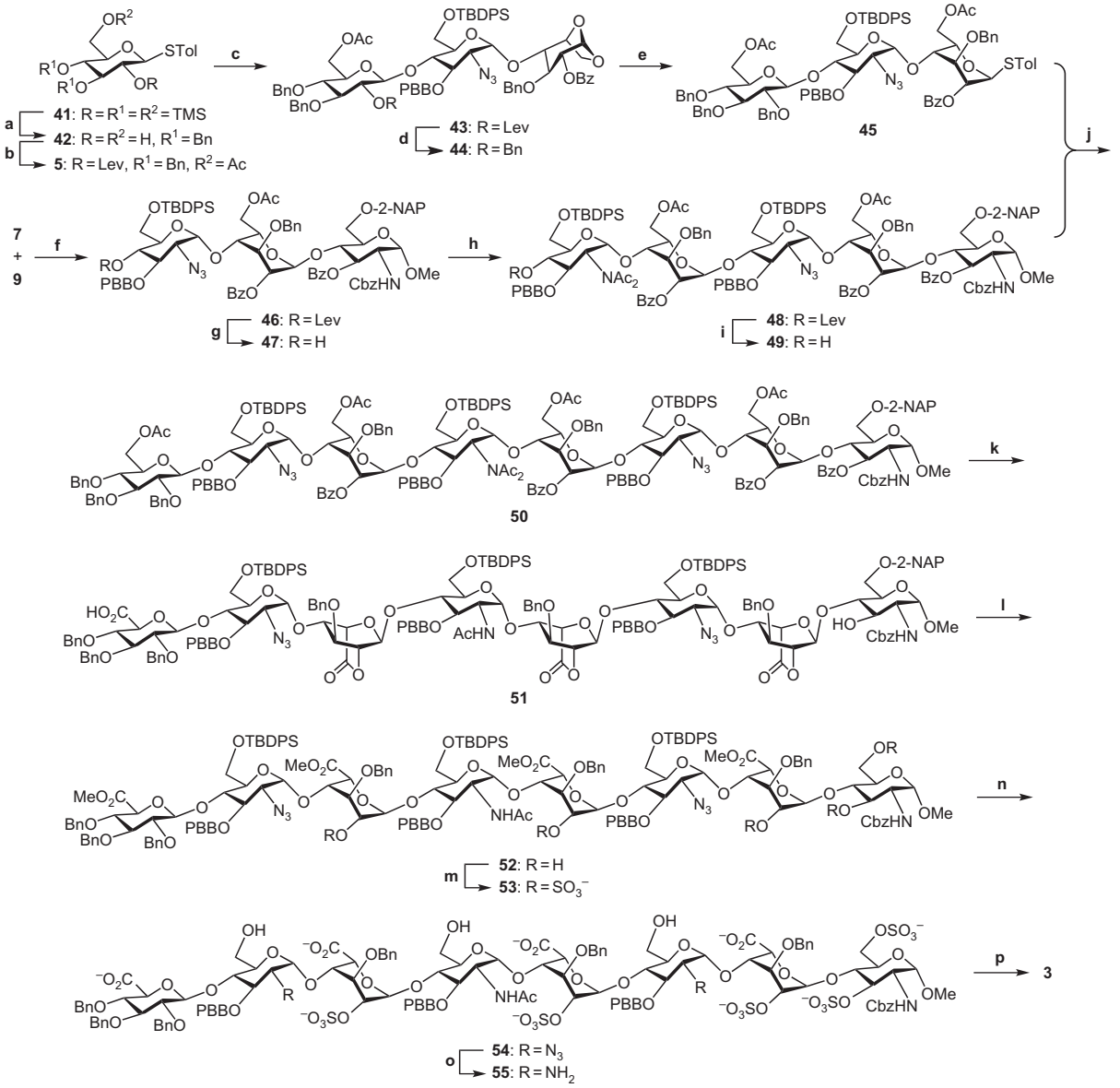

Figure 5 | Synthesis of target octasaccharide 3. a, Benzaldehyde, TMSOTf, CH_2Cl_2, −78 °C, 2 h; benzaldehyde, Et_3SiH, TMSOTf, −78 °C, 4 h; BH_3·THF, TMSOTf, 5 h, 86% (one pot); **b**, (1) Ac_2O, Et_3N, CH_2Cl_2, 83%; (2) Lev_2O, Et_3N, DMAP, CH_2Cl_2, 98%; **c**, **6**, TfOH, NIS, CH_2Cl_2, −78 °C → −20 °C, 3 h, 92%; **d**, (1) NH_2NH_2, acetic acid, Pyr, 5 h, 93%; (2) NaH, BnBr, CH_2Cl_2/DMF (30/1, v/v), 0.5 h, 82%; **e**, (1) $Cu(OTf)_2$, Ac_2O, 88%; (2) TMSSTol, ZnI_2, CH_2Cl_2, 3 h, 95%; **f**, TMSOTf, CH_2Cl_2, −40 °C, 3 h, 70%; **g**, NH_2NH_2, AcOH, Pyr, 5 h, 85%; **h**, **8**, NIS, TfOH, CH_2Cl_2, −78 °C → −20 °C, 4 h, 91%; **i**, NH_2NH_2, AcOH, Pyr, CH_2Cl_2, 1.5 h, 81%; **j**, NIS, TfOH, CH_2Cl_2, −78 °C → −20 °C, 4 h, 89%; **k**, (1) NaOMe, MeOH/CH_2Cl_2 (1/1.3, v/v), 85%; (2) TEMPO, BAIB, CH_2Cl_2/H_2O (1/1, v/v), 80%; **l**, (1) DDQ, CH_2Cl_2/H_2O (18/1, v/v), 3.5 h, 71%; (2) LiOH, THF, 1 h; (3) CH_2N_2, CH_2Cl_2, Et_2O, 81% (two steps); **m**, SO_3·Et_3N, DMF, 60 °C, 76%; **n**, (1) HF·Pyr, Pyr, 83%; (2) LiOH, H_2O_2, H_2O, THF, MeOH, 37 °C, 74%; **o**, 1,3-propanedithiol, Et_3N, Pyr/H_2O (4/1, v/v), 50 °C, 81%; **p**, (1) SO_3·Pyr, $NaOH_{(aq)}$, Et_3N, MeOH; (2) $Pd(OH)_2$/C, H_2, phosphate buffer pH = 7, 47% (two steps).

deacetylation to provide the corresponding 1-alcohol in 76% yield. Other acetyl groups were presumably cleaved in this reaction, causing a yield drop in comparison with the preparation of donors **14** and **7**. Reaction of this hemiacetal with CCl_3CN and K_2CO_3 led to the expected TCI donor **29**. We next turned our attention to the synthesis of the reducing end sugar unit **16**. Methyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**30**) was prepared in 49% overall yield through a six-step protocol starting from *N*-acetyl-D-glucosamine (see Supplementary Information). Regioselective installation of TBDPS in **30** led to 4-alcohol **16** (92%), which was glycosidated by donor **29** under TMSOTf activation to give heptasaccharide **31** in 76% yield.

An alternative coupling procedure was also studied. Transformation of the 1-*C*-acetate of the hexasaccharide **28** to the thioether was carried out by treatment with TMSSTol and ZnI_2, furnishing the thioglycoside **32** in 92% yield. The expected heptasaccharide **31** was acquired in 76% yield through the condensation of **32** and **16** in the presence of NIS and TfOH. This two-step procedure is shorter than the TCI route, with an improved 70% overall yield from compound **28**. Cleavage of 2-NAP in **31** by DDQ generated the heptasaccharide acceptor **33**.

Preparation of the target molecule **4** is summarized in Fig. 4. The non-reducing end L-*ido* sugar **13** was prepared through 4-*O*-benzylation (Ag_2O, benzyl bromide) of alcohol **12**, forming ether **34** followed by the usual acetolysis–deacetylation–imidation protocol. On TMSOTf activation, coupling of **13** and acceptor **33** gave the fully protected octasaccharide **35** in 78% yield. Alternatively, a one-pot [1 + 6 + 1] glycosylation was investigated. First, the 2-NAP group

of thioglycoside **32** was cleaved to provide alcohol **36**. TfOH-promoted coupling of **13** with **36** followed by addition of alcohol **16** in the presence of NIS and TfOH afforded the octasaccharide **35** in a moderate yield of 31%.

With compound **35** in hand, a series of functional group transformations was carried out. Removal of all ester groups using sodium methoxide gave alcohol **37** (78%), which underwent regioselective 2,2,6,6-tetramethyl-1-piperidinyloxyl free radical (TEMPO)-oxidation of the primary hydroxyl groups to yield lactone **38** (68%). The structure of **38** was confirmed by several two-dimensional nuclear magnetic resonance (NMR) experiments, indicating the significant downfield shifts of the 2-H signals on all four L-*ido* rings (see Supplementary Information). Desilylation by tetra-*n*-butylammonium fluoride in the presence of acetic acid followed by lactone ring opening with LiOH supplied the corresponding carboxylate **39**. Per-*O*-sulfonation of all hydroxyls with sulfur trioxide triethylamine complex (SO_3·Et_3N) led to the corresponding sulfate derivative **40** in 62% yield. Hydrogenolysis was carried out to cleave all Bn and PBB groups simultaneously with the conversion of the azido into the amino groups. The amino-alcohol was subjected to *N*-sulfonation with sulfur trioxide pyridine complex (SO_3·Pyr) through pH control at ~9.5 to afford the crude product **3**. Purification through Sephadex G25 and ion-exchange with Dowex 50WX8-Na^+ resin furnished the target molecule **4** in 72% yield from **40**. Analysis of one- and two dimensional NMR spectra (see Supplementary Information) confirmed the structure of compound **4**. High-resolution electrospray ionization–mass spectrum (ESI-MS) gave a m/z value of 871.5614 corresponding to $[M + 9Na–12H]^{3-}$ and the formula $C_{49}H_{71}N_4O_{80}S_{13}Na_9$ (calculated value, 871.5608).

Synthesis of compound 3. Figure 5 depicts the synthetic method used for the acquisition of compound **3**. The per-*O*-trimethylsilylated thioglycoside **41** was converted to the 2,6-diol **42** in an excellent 86% yield via the TMSOTf-promoted one-pot process[30–32], which included 4,6-*O*-benzylidene formation, 3-*O*-benzylation and 6-*O*-ring opening. Regioselective 6-*O*-acetylation followed by 2-*O*-levulinylation generated the non-reducing end monosaccharide synthon **5**, which was coupled with the glycosyl acceptor **6** under NIS/TfOH promotion to furnish the trisaccharide **43** (92%) as a single isomer. The 2″-*O*-Lev group, used only in driving the 1,2-*trans* stereoselective glycosidation, needs to be replaced in consideration of the final structure. Thus, hydrazine treatment of **43** led to the 2″-alcohol (93%), which was benzylated under the NaH/benzyl bromide tandem in a CH_2Cl_2/DMF (30/1, v/v) mixed solvent to afford the ether **44** (82%). In this etherification, the solvent combination, 30 min reaction time and acetic acid quenching assisted in preserving the installed ester moieties. The 1,6-anhydro ring of **44** was then opened by acetolysis, and subsequent reaction with TMSSTol and ZnI_2 brought about the formation of the thioether **45**.

The 4-alcohol **9** was prepared from the known methyl 2-*N*-benzyloxycarbonyl-2-deoxy-α-D-glucopyranoside[36] in three steps, with an overall yield of 70% (see Supplementary Information). Condensation of this acceptor with the TCI donor **7** delivered trisaccharide **46**, which underwent hydrazine-mediated Lev cleavage to afford acceptor **47**. Further coupling with thioglycoside **8** under NIS/TfOH activation led to pentasaccharide **48** (91%). Our attempts at generating this glycosidic linkage gave very low yields when we used different lengths of TCI and STol donors having trifluoroacetyl as the protecting group for the amine destined for acetylation. Complete replacement of all hydrogen-bonding protons in the amine by acetyl groups favoured the coupling reaction. Hydrazinolysis of **48** supplied alcohol **49**, which was condensed with glycosyl donor **45** to obtain the desired octasaccharide **50** in 89% yield.

Compound **50** was next subjected to functional group transformations *en route* to the final compound. Saponification of **50** resulted in cleavage of all *O*-acyl (Bz and Ac) groups and the conversion of NAc_2 into NHAc, fashioning a polyhydroxylated compound (85%) that, on TEMPO oxidation, gave the lactone and carboxylate derivative **51** in 80% yield. DDQ-promoted removal of 2-NAP provided the corresponding 6-alcohol, which underwent successive lactone ring opening with LiOH, and methylation of the generated carboxylate groups using diazomethane furnished compound **52**. The material obtained after LiOH treatment was difficult to purify, so methyl ester formation was deemed necessary to afford a homogeneous compound for *O*-sulfonation, a critical step in the transformation process. Treatment of pentaol **52** with SO_3·Et_3N smoothly generated the desired sulfate derivative **53** in 76% yield after Sephadex LH-20 column purification. The TBDPS and methyl ester functionalities were then consecutively removed, supplying product **54**. To allow *N*-sulfonation, the azido groups were selectively reduced to the free amine by 1,3-propanedithiol and the desired derivative **55** was obtained in 81% yield. Immediate sulfonation of the amino groups using SO_3·Pyr in the presence of Et_3N and NaOH followed by global hydrogenolysis finally delivered target molecule **3** in 47% yield from diamine **55**, after gel filtration and Na^+ cation exchange. As with compound **4**, the structure of **3** was analysed and supported by one- and two-dimensional NMR experiments together with high-resolution ESI-MS measurements (see Supplementary Information).

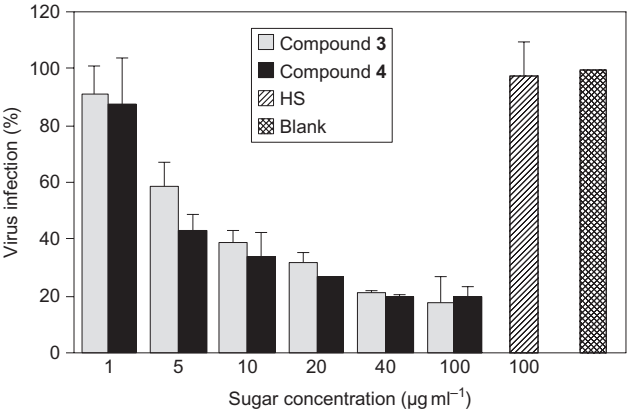

Figure 6 | Inhibition of HSV-1 infection of Vero cells by compounds 3 and 4. Vero cells were infected with HSV-1 in the absence or presence of either octasaccharide **3** or **4** for 60 min on ice. A commercially acquired HS was also tested for comparison. The cells were then washed with phosphate-buffered saline and overlaid with 1% agarose for plaque determination assays as described in the Methods. The number of plaques obtained in the absence of sugars was assigned as 100% and referred to as blank. The results displayed are means of duplicate batches, each batch carried out in two trials. Error bars are standard deviations of the means of the individual batches.

Inhibition of viral infection. The capability of the synthesized compounds in disrupting HSV-1 infection was next investigated. HSV-1 was mixed with various concentrations of octasaccharides **3** and **4**, and a commercially acquired HS was also tested for comparison. The resulting mixtures were used to infect Vero cells at 37 °C for 60 min, and plaque numbers were counted 3 days after infection. Both octasaccharides reduced plaque formation in a dosage-dependent manner and gave strikingly similar inhibition profiles (Fig. 6). Approximately 80% of the infection was inhibited at 40 μg ml^{-1} and at 100 μg ml^{-1}; this closely resembles the results for compound **2** (40 μM) inhibition of HSV-1 infection of HeLa cells[18]. The half-maximal inhibitory concentrations (IC_{50}) for compounds **3** and **4** were determined to be 5.4 μg ml^{-1} and 3.9 μg ml^{-1}, respectively. In contrast, no significant inhibition was observed with commercial HS at 100 μg ml^{-1}. These results

clearly demonstrate that the synthesized 3-*O*-sulfonated HS octasaccharides competed with cell surface HS and blocked HSV-1 infections of Vero cells. It has been shown previously that enzymatically generated compounds **1** and **2** have nearly identical binding affinity with HSV-1 gD (refs 17, 18). These findings, and our own data, suggest that differences in HS fine structures and the location of the requisite 3-*O*-sulfonate group do not have a sizeable effect on the extent of inhibition of HSV-1 infection, or on gD binding for that matter. These assumptions therefore offer greater flexibility in designing 3-*O*-sulfonate-containing HS oligosaccharides that specifically prevent gD-mediated HSV-1 entry into the host cell.

Conclusions

We have successfully developed a convenient route for the synthesis of the specific 3-*O*-sulfonated octasaccharides **3** and **4** corresponding to the previously suggested gD-binding structures. The preparation made use of key disaccharide intermediate **10**, which was effectively converted into various building blocks. Overall, this is the first report of irregular HS octasaccharides acquired through chemical synthesis. The *in vitro* inhibition of HSV-1 infection exhibited by these oligosaccharides is a step towards a better understanding of viral attachment and entry. Interaction between these sugars and HSV-1 glycoprotein gD, and particularly the complex structure at the molecular level, will be further studied to provide valuable information for the discovery of new anti-HSV-1 drugs.

Methods

Inhibition of HSV-1 infection by octasaccharides 3 and 4. Vero cells were seeded at a density of 2×10^5 cells per well in six-well plates. Next day, the synthesized sugars (compounds **3** or **4** at 1, 5, 10, 20, 40 and 100 $\mu g\ ml^{-1}$) or a commercially acquired HS (Sigma, from bovine kidney, 100 $\mu g\ ml^{-1}$) were mixed with 200 plaque-forming units per well of HSV-1 (KOS) tk12 (ref. 37) recombinant virus on ice for 30 min. The mixtures were then added to Vero cells on ice for 1 h. The infected cells were subsequently washed with phosphate-buffered saline and overlaid with 1% agarose at 37 °C for 3 days. The cells were then fixed with 10% formaldehyde at room temperature for 15 min and stained with crystal violet to visualize plaques for counting. The numbers of plaques obtained in the absence of compounds **3** or **4** were assigned as 100%.

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Author contributions

S-C.H. conceived the idea of HS synthesis, supervised students to carry out the experiments, drew and summarized the figures, and finalized the preparation of the manuscript. Y-P.H. and S-Y.L. synthesized the irregular HS octasaccharides **3** and **4**, respectively. C-Y.H. carried out the inhibition experiments of HSV-1 with Vero cells. M.M.L.Z. participated in the discussion and wrote the manuscript. J-Y.L. initiated the work on the preparation of the oligosaccharide skeleton. W.C. supervised C-Y.H. on the inhibition study of HSV-1 infection.

Additional information

The authors declare no competing financial interests. Supplementary information and chemical compound information accompany this paper at www.nature.com/naturechemistry. Reprints and permission information is available online at http://www.nature.com/reprints/. Correspondence and requests for materials should be addressed to S.C.H.