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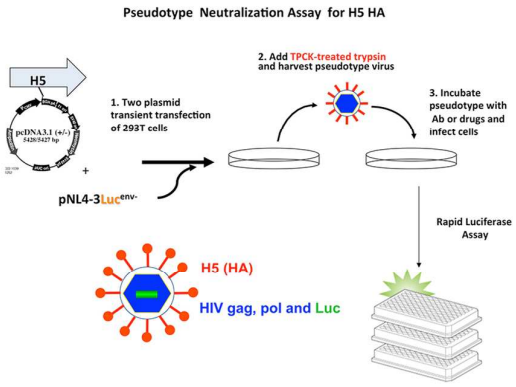

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CONCISE ARTICLE

Inhibition of Influenza H5N1 invasion by modified heparin derivatives

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Influenza remains a serious health threat, with resistance to frontline drugs becoming more common, and new treatments urgently sought. One strategy for the inhibition of the attachment of influenza to host cells is to employ chemically modified heparins, capable of effectively competing with the multivalent interactions involved. In an assay of H5N1 influenza viral invasion comprising a H5 pseudotyped HIV system, selective removal of the sulfate groups from heparin (IC_{50} ~ 22 x 10^{-9} g.mL^{-1}) allowed the retention of inhibitory activity in the products (IC_{50} ~ 4 x 10^{-9} g.mL^{-1}) while significantly reducing their anticoagulant activities. Chemically modified anionic polysaccharides offer a potential source of effective inhibitors of viral attachment, which are suitable for further optimisation.

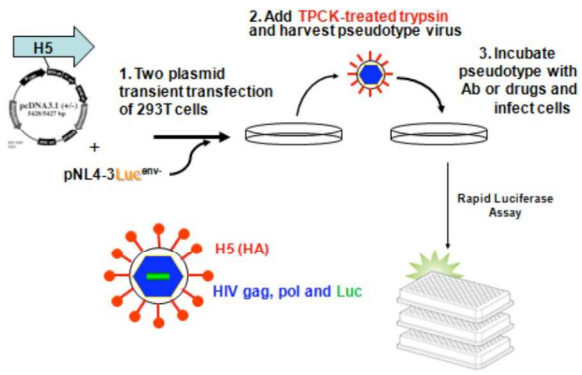
1. Introduction

Influenza is an infectious disease, known to affect both birds and mammals [1], caused by RNA viruses of the *orthomyxoviridae* family. Transmission occurs through direct contact, interaction with fomites, and/or by the inhalation of aerosols containing viral particles. Symptoms of the disease commonly include chills, fever, sore throat, muscle soreness, headaches, coughing, weakness and fatigue, with more severe cases leading to potentially life threatening pneumonia, especially amongst the young and elderly.

Three genera of influenza affect humans, namely influenza A, B and C [2], the influenza A genus being responsible for significantly higher degrees of morbidity and fatality. Influenza B, uncommon in comparison to influenza A, exhibits a low mutation rate that impairs genetic diversity and prevents this genus from causing pandemics [3]. Influenza C is the rarest form, generally causing only minor illness in juveniles [4, 5].

Influenza A is ordinarily classified into strains based on the immunogenic properties of the haemagglutinin (HA) and neuraminidase (N) glycoproteins present on the viral coat [6]. The haemagglutinin molecules are thought to assist viral entry by forming complexes with sialic acid residues [7-9], while the neuraminidase protein hydrolyses distinct residues present within the sialic acid receptor, liberating newly synthesised viral progeny [10]. Neuraminidase may also be implicated in the infection of the lung epithelium [11]. Sequence heterogeneity exists between strains for these proteins but, in general, the architecture of the active sites remains comparable [12, 13].

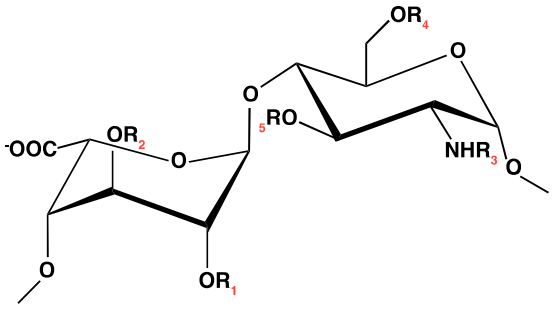
Avian influenza, H5N1, is a highly pathogenic influenza A virus that infects migratory birds and poultry in Asia, Europe and Africa. H5N1 influenza is characterised by higher infection and mortality rates (~60 % in 2009; with 262 deaths out of 442 cases [14]) compared to other serotypes. High viral load, combined with an intense inflammatory reaction, is thought to contribute significantly to the pathogenesis of the disease [15]. Avian to human transmission of this subtype was first documented in 1997 and, since then, progressively higher rates of cross-species transmission have been reported [16], suggesting a persistent threat of human H5N1 influenza pandemic. Previous pandemics in 1918 (H1N1), 1957 (H2N2), 1968 (H3N2) and the 2009 (H1N1) "swine" flu have caused an accumulated 50 million deaths worldwide with massive socio-economic consequences. To this end, large sums of money are being invested in seeking a better understanding of epidemiology, methods of containment, and on the development of novel therapeutics and treatments.



Scheme 1. *The H5-pseudotyped-HIV assay.*

In addition to vaccines against influenza, which comprise the major prophylaxis strategy, but suffer from the unpredictability of emerging strains with pandemic potential, long manufacturing times and expense, there are two distinct classes of antiviral agents licensed for use in the treatment of influenza. These are the M2 inhibitors (or adamantanes) and the neuraminidase inhibitors. The M2 inhibitors (e.g. amantadine and rimantadine) obstruct the M2 viral ion channel protein and are specific to members of the influenza A genus. This class of

antivirals is commonly used for protection in poultry farms and is useful only in the initial stages of infection. Resistance to M2 inhibitors is generally high (91 % for the 2005 H3N2 strain), with contra-indications for persistent and uncontrolled use. Neuraminidase inhibitors, on the other hand, have generally been the product of structure-based drug design and prevent viral reproduction by inhibition of the budding process required to release new progeny. Examples include Zanamivir, Oseltamivir and Peramivir (currently licensed for emergency intravenous use). Resistance to neuraminidase inhibitors has also been observed widely [17].



Scheme 2. *Predominant repeating disaccharide of heparin and heparin derivatives*; α-L-iduronic acid 1-4 linked to α-D-glucosamine. R_1, R_2, R_4 and R_5 = OH/SO_3^-, R_3 = H/NAc/ SO_3^-. See **Table 1** for the substitution patterns of the chemically modified heparins used in this manuscript.

Despite the current drawbacks of these antivirals, which include a lack of parental agents from which diverse derivatives can be generated for use in critical patients, increased resistance, diminished treatment efficacy and the post-treatment emergence of anti-viral resistance (for H5N1 disease in humans), governments have recently stockpiled reserves of zanamivir and oseltamivir as first line treatments. Furthermore, resistance to oseltamivir has been demonstrated in H5N1 infected patients in Vietnam [18]. Hence, there remains a pressing need to develop novel pharmaceutical agents to assist the fight against future influenza outbreaks. In this regard, inhibitors of virion attachments to the cell membrane have the advantage of preventing the earliest phase of influenza infection. Indeed, the drug DAS181 that selectively cleaves sialic acids from the cell host is being tested in Phase II clinical trials [19], however, additional compounds that inhibit viral entry are highly desirable for either the prevention or treatment of influenza infection.

The ability to screen potential therapeutic agents experimentally has been enhanced by the routine use of retroviral pseudotype systems, permitting safe evaluation in a desirable high-throughput format, without the need for "live" viruses. These pseudotypes encode reporter genes and bear foreign viral envelopes of interest. Indeed, the retroviral and lentiviral pseudotypes have been used previously for studies on highly pathogenic viruses such as influenza [20], SARS [21] and Ebola [22]. In this study we have pseudotyped the human immunodeficiency virus type 1 (HIV-1) pNL4-3 vector devoid of the HIV env gene with the HA gene from an avian H5N1 isolate. A sensitive lentiviral pseudotype assay to screen compounds for their ability to inhibit viral invasion, has thereby been established (**Scheme 1**).

The polyanionic carbohydrates heparin and dextran sulfate were shown to be highly potent inhibitors of viral invasion; the latter exhibiting size-dependent activity [23]. Pharmaceutical grade heparin, a sulfated polyanion, is available widely because it forms one of the mainstays of anticoagulant treatment in the clinic. Heparin chains comprise a repeating disaccharide backbone subunit consisting predominantly of an L-iduronic acid residue covalently attached to N-sulfo D-glucosamine (**Scheme 2**). The backbone is heavily decorated with sulfate groups (O-linked) at C-2 of the iduronic acid and C-6 of the N-sulfoglucosamine residues. Heparin polysaccharides are bio-synthesised exclusively by mast cells and most commonly extracted from porcine mucosa. Other sulfated polyanions, such as dextran sulfate, may also possess considerable anticoagulant activity and this has tended to preclude other clinical applications for these polysaccharides.

Table 1. *Sulfation pattern of the 9 chemically modified heparin derivatives.*

Polysaccharide	Repeating Disaccharide	R1 (I-2)	R2 (I-3)	R3 (A-2)	R4 (A-6)	R5 (A-3)
1	IdoA(2S)-GlcNS(6S)	SO^-_3	OH	SO^-_3	SO^-_3	OH
2	IdoA(2S)-GlcNAc(6S)	SO^-_3	OH	NAc	SO^-_3	OH
3	IdoA-GlcNS(6S)	OH	OH	SO^-_3	SO^-_3	OH
4	IdoA(2S)-GlcNS	SO^-_3	OH	SO^-_3	OH	OH
5	IdoA-GlcNS	OH	OH	SO^-_3	OH	OH
6	IdoA-GlcNAc(6S)	OH	OH	NAc	SO^-_3	OH
7	IdoA(2S)-GlcNAc	SO^-_3	OH	NAc	OH	OH
8	IdoA-GlcNAc	OH	OH	NAc	OH	OH
9	IdoA(2,3S)-GlcNS(3,6S)	SO^-_3	SO^-	SO^-_3	SO^-_3	SO^-_3

The selective chemical modification of heparin polysaccharides has been used successfully to decouple favourable bioactivity from adverse off-target interactions for potential applications in the treatment of Alzheimer's disease and severe malaria [24, 25]. Several studies have shown that unmodified heparin can serve as an effective inhibitor of viral entry, examples are dengue fever in human liver cell lines [26], murine leukemia virus [27], herpes simplex virus [28], yellow fever [29] and human T-lymphotropic virus [30]. Here, we explore the potential of heparin and chemically modified heparin derivatives (**Table 1**) against avian influenza (H5N1) invasion. An attractive strategy would be to tailor chemically modified heparins so that they retain their selective inhibitory activity against influenza viral invasion, while their undesirable anticoagulant activities are ameliorated.

2. Experimental

2.1 Materials. Porcine intestine mucosal heparin (201 $IU.mg^{-1}$) was obtained from Celsus Laboratories, Cincinatti, Ohio, USA. All chemicals and solvents required for chemical modification of the polysaccharides were purchased from Alfa Aesar

(Lancashire, UK) except for HPLC grade water, which was obtained from Fisher Scientific (Loughborough, UK).

2.2 Chemically modified heparin preparation. The synthesis of the chemically modified polysaccharides, starting from the parental compound, porcine mucosal heparin (M_w = 12 kDa) (**Table 1** and **Scheme 2**) is described in the electronic supplementary information (ESI) available online†. The effectiveness of all chemical modifications was monitored using ^1H and ^{13}C NMR at 500 and 125 MHz respectively (in D_2O, 27 °C). Chemical shifts, δ (ppm) (compared to TSP as an external reference standard) were in full accord with well-defined reference compounds [31].

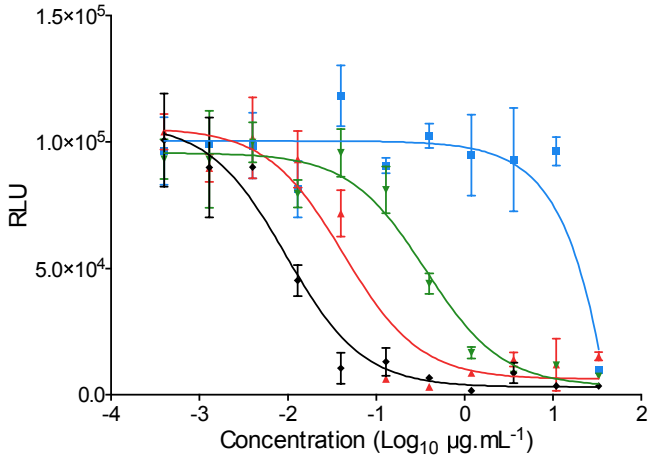


Figure 1. *Inhibition of H5-pseudotyped-HIV invasion of 293T cells by anionic polysaccharides.* The inhibition curves of the H5-pseudotyped-HIV invasion assay in the presence of 3-fold serial dilutions of heparin (**P1** – black, diamond), persulfated heparin (**P9** – red, inverted triangle), totally desulfated heparin (**P8** – blue, square) and locust bean gum sulfate (**LBGS** – green, upside down triangle) are shown. **P1** inhibited H5-pseudotyped-HIV with an IC_{50} ~ 20 ng.mL^{-1} whereas **P9** inhibited at ~ 42 ng.mL^{-1}.

2.3 Cell culture. 293T kidney epithelial cells were maintained in DMEM supplemented with 2 mmol.L^{-1} glutamine, penicillin (100 U.mL^{-1}), streptomycin (100 µg.mL^{-1}; BioWhittaker), and 10 % fetal bovine serum (FBS; HyClone Europe, Ltd, Cramlington, UK) (complete DMEM). For transfections, cells were plated at 6 x 10^5 cells.mL^{-1} in 75 cm^2 flasks in DMEM supplemented with 2 mmol.L^{-1} glutamine, penicillin (100 U.mL^{-1}), streptomycin (100 µg.mL^{-1}), and 2 % foetal bovine serum. For pseudovirus infectivity assays, cells were plated at 2.5 x 10^5 cells per well on 96-well microplates.

2.4 Plasmids. For cloning avian H5 into an expression vector, total H5N1 cDNA was kindly provided by Dr. Ilaria Capua (Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy). The H5 coding sequence (Influenza A virus A/*Cygnus olor*/Italy/742/2006; HA NCBI Acc. No. CY017035) was initially amplified from total cDNA by PCR using the following primers: 5'-ATGGAGAAAATAGTGC TTCTTCTTGC-3' and 5'- TTAAATGCAAATTCTGCATTG TAACGACCC-3' and cloned into the pCR®2.1- TOPO® vector (invitrogen) according to manufacturer's instructions. The H5 coding sequence was subsequently amplified from the pCR®2.1- TOPO® vector by PCR using the following primers: 5'- ATATAAGCGGCCGCCATGGAG AAAATAG TGCTTCT-3' and 5'- ATATAACTCGAGTTAAATGCAA ATTCTGCATTG-3' and cloned into the pcDNA3.1(+) vector (Invitrogen). The pNL4-3.Luc.R-/-Env- contains two frameshifts that render the pNL4-3 infectious molecular clone Env- and Vpr- whereas the nef-encoding region is replaced with either Luciferase (Luc) reporter gene (NIH AIDS Research and Reference Program, Division of AIDS, NIAID, NIH from Dr. Nathaniel Landau).

2.5 Production of H5-pseudotyped HIV virions. 293T kidney epithelial cells were co-transfected with the H5-expressing pcDNA3.1-H5 and the pNL4-3.Luc.R-Env- by Lipofectamine 2000 (Invitrogen, S.R.L, San Giuliano Milanese, Italy) according to the manufacturer's instructions. For production of positive control VSV-G pseudotyped virions, 293T cells were co-transfected with pMD2.G, a CMV-driven expression plasmid that encodes the vesicular stomatitis virus VSV-g envelope protein, and the pNL4-3.Luc.R-E- plasmids.

After 24 hrs of incubation at 37 °C, medium was replaced with DMEM supplemented with 2 mmol.L^{-1} glutamine, penicillin (100 U.mL^{-1}), streptomycin (100 µg.mL^{-1}), and 0.13 mg.mL^{-1} L-(tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK)-treated trypsin (Worthington Biochemical Corporation, Lakewood, NJ, USA), or complete DMEM for VSV-g pseudotype viral production. Virion containing supernatants were harvested after 24 h, centrifuged and filtered through a MILLEX®-HV PVDF 0.45 µm filter (Millipore, Co. Cork, Ireland).

2.6 Titration of H5-pseudotyped-HIV viral stock. H5-pseudotyped viral stock was titrated on 293T cells as follows: 2-fold serial dilutions of the virus were transferred to 293T cells (37 °C). Luciferase activity was measured 48 h post infection according to the manufacturer's instructions (Luciferase assay system, Promega, Madison, Wisconsin, USA). TCID50 was calculated using a non-linear regression curve (Prism GraphPad software v. 4.0., San Diego, CA, USA) prior to conversion into IU.mL^{-1} according to the Reed-Muench formula [32].

2.7 Microneutralization Assay. Briefly, 192 IU of VSV-G-pseudotyped or H5-pseudotyped viral supernatants were incubated in the presence of 5-fold serial dilutions of the test compound, in a 1:1 ratio, and incubated for 30 min. at room temperature, prior to addition to 293T cells. Luciferase activity was measured 48 h after infection according to the manufacturer's instructions (Luciferase assay system, Promega, Madison, WI, USA).

2.8 Anti-Factor Xa activity. The anti-Factor Xa activity was measured against the parental porcine intestine mucosal heparin precursor from which the chemically modified heparin polysaccharides were derived. The anti-FactorXa activity of this heparin was established previously as 201 IU.mg^{-1}. Relative anticoagulation potential was determined using a clinical diagnostic grade Coatest Heparin test kit (Chromogenix, MA), that was previously adapted to a 96-well plate format, reading absorbance at λ = 405 nm (MultiScan EX plate reader (Thermo Fisher Scientific, Loughborough, UK)).

2.9 Prothrombin time (PT) anticoagulation activity. PT measurements were conducted for bioactive polysaccharides by modification of the manufacturer's procedure [Siemens Diagnostics Thromborel™ S]. Briefly, human plasma (normal) was obtained from whole blood post collection (into sodium citrate) by centrifugation at 1500 g for 15 min. Human plasma (room temperature) was added to either a water control (100 µL) or heparin/modified heparin (100 µL) and incubated for 1 min at 37 °C in a pre-incubated tube. Immediately, pre-incubated (37 °C) Thromborel™ S reagent (100 µL of 2-fold manufacturer's concentration) was added to the mixture, the tube manually agitated, and the time required for clot formation was noted.

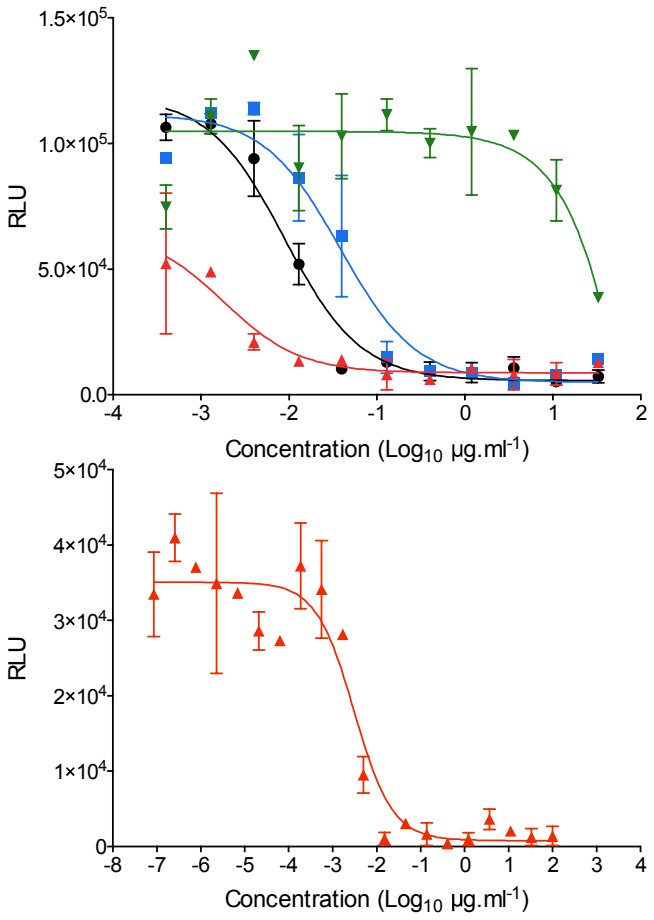


Figure 2. *Chemically modified heparins retain the ability to inhibit viral invasion.* (Upper) Inhibition curves of the H5-pseudotyped-HIV invasion assay in the presence of 3-fold serial dilutions of the most effective inhibitors; heparin (**P1** – black, circle), N-acetylated heparin (**P2** – blue, square), 2-O-desulfated heparin (**P3** – red, triangle) and one weaker inhibitor, 6-O-desulfated heparin (**P4** – green, upside down triangle). (Lower) A titration of the most effective inhibitor, 2-O-desulfated heparin (**P3** – red, triangle) to lower concentrations.

2.10 Activated partial thromboplastin time (aPPT) anticoagulation activity. aPPT measurements were conducted for bioactive polysaccharides by modification of the manufacturer's procedure [Siemens Diagnostics Pathromtin™ SL]. Briefly, human plasma (normal) was obtained from whole blood post collection (into sodium citrate; 11 mM final concentration) by centrifugation at 1500 g for 15 minutes. Human citrated plasma (100 □L, 20 °C), a water control (50 □L) or heparin/modified heparin test sample (50 □L) and Pathromtin™ SL reagent (100 □L, 20 °C) were added to a pre-incubated tube (37 ° C). The tube contenets were mixed by tilting prior to incubation (37 °C, 2 min). Pre-incubated calcium chloride (50 □L, 50 mM, 37 ° C) was added to the tube before manual agitation commenced and the time required for clot formation noted.

3. Results and Discussion

3.1 Heparin inhibits influenza A H5N1 pseudotype viral invasion. It is clear from **Figure 1** that viral entry can be inhibited to different extents with a range of anionic polysaccharides (**Table 1** and **Scheme 2**). The inhibition potential of heparin (**P1**) determined using the pseudotype assay, with an IC_{50} of 20 ng.mL^{-1}, is broadly comparable with previous work conducted on the live influenza A virus [33, 34] and (influenza) viral derived neuramindase assays [35] with IC_{50} values of 18 and 50 ng.mL^{-1} respectively, despite the different experimental systems employed.

It should be noted that this viral inhibition is not achieved solely by high charge density, as can be seen from the inhibition data presented for per-sulfated heparin (**P9**) [IC_{50} (**P9**) = 42 ng.mL^{-1}] (**Figure 1**). The chemical O-sulfation of free hydroxyl groups in **P9** leads to a significantly higher degree of sulfation and overall negative charge compared to that of the parental **P1** but is less active. Other non-GAG sulfated polysaccharides also possess inhibitory activity, for example locust bean gum sulfate (**LBGS**, **Figure 1**) and dextran sulfate, as previously reported by Lüscher-Mattli *et al.* [36]. However, moderate sulfation is necessary for potent inhibition; the chemically de-sulfated heparin polymer (**P8**) (devoid of all N- and O- sulfation) only weakly inhibits viral invasion. Polysaccahride **P8** possesses only one carboxylic acid group per constituent disaccharide repeat unit and demonstrates bio-activity that is 2,000-fold less than that of heparin. In order to explore specificity, some of the best performing compounds, including unmodified heparin, were tested for their capacity to inhibit reporter viruses pseudotyped with the Vesicular Stomatitis virus glycoprotein (VSV-g) instead of H5. As expected, none of the compounds were able to inhibit VSV-g reporter virus infection, even at high concentrations (**Supplementary Figure 1**).

3.2 Chemically modified heparins retain the ability to inhibit viral invasion. **Figure 2** (upper) shows that removal of one sulfate group per disaccharide unit (**Table 1**) has a variety of effects; diminishing activity in **P2** (10 x) and drastically reducing it in the case of **P4**. Notably, **P3** has significantly improved inhibitory potency (**Figure 2** Upper and Lower), despite having a lower sulfation level than its parent, **P1,** and a sulfation level comparable to **P2** and **P3**. This result confirms that the effect is not driven solely by charge density and suggests that conformational considerations are influencing activity. It is also notable that several modified polysaccharides retain the ability to inhibit viral invasion, at least to some extent, while having quite distinct substitution patterns and conformational properties. A level of redundancy does therefore seem to be at play in this system.

Recent structural investigations have revealed that single modifications to the heparin backbone at the amino group of glucosamine (N-sulfation to N-acetylation as in **P2**) or, at position-2 of iduronate (as in **P3**), result in distinct conformational change [37, 38].

In contrast, removal of the O-sulfate at position-6 of glucosamine residues (as in **P4**) has a more modest effect on the conformation (predominantly at the bond between position-4 of glucosamine and the glycosidic oxygen). In all cases, removal of more than one sulfate group renders the polysaccharide essentially unable to disrupt viral invasion. The results suggest that appropriate charge distribution and conformation, rather than overall charge or charge density, control the inhibition of viral entry.

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Table 2. *Inhibition of Infection of epithelial 293T cells with the H5-pseudotyped-HIV by heparin derivatives as IC_{50} values together with anticoagulant activities (anti-factor Xa, PT and aPTT assays).*

Polysaccharide	H5N1 IC_{50} (ng.mL^{-1})	ATIII:Factor Xa (%)	cPT (s^{-1})	aPTT (s^{-1})
1	22.39	100.00	55	6.7
2	60.24	0.03	> 1000	166.7
3	4.21	0.40	> 1000	>1000.0
4	>1000.00	0.50	500	16.7
5	>1000.00	0.03	> 1000	>1000.0
6	>1000.00	0.03	> 1000	>1000.0
7	>1000.00	0.03	> 1000	>1000.0
8	>1000.00	0.03	> 1000	>1000.0
9	26.02	35.00	20	>1000.0

3.3 Anticoagulation activity of modified polysaccharides. Parameters relating to the anticoagulant properties of the polysaccharides can be found in **Table 2**. The antithrombin (ATIII):Factor Xa activity of all the chemically modified heparin polysaccharides are highly attenuated compared to **P1** (≤ 0.5 %), with the exception of **P9**, the over-sulfated form of heparin. This compound was included to explore the consequences of higher charge density, and exhibited activity 35 % that of **P1**, even though **P9** is more highly sulfated than **P1**. Sulfation level is not, therefore, directly proportional to the ATIII:Factor Xa activity. All desulfated heparin derivatives exhibited minimal extrinsic activity (prothrombin time, PT) compared to **P1** while, in contrast to ATIII:Factor Xa activity, **P9** displayed increased activity (2.5-fold) compared to **P1** (**Table 2**).

The anticoagulant cascade consists of three main processes, involving the interplay of several protein factors. It is convenient to divide these into three groups, termed the intrinsic, extrinsic and common pathways, which can be examined by separate assays; the activated partial thromboplastin time (aPTT), the prothrombin time (PT) and the anti-factor Xa activity assays. The aPTT assay measures the intrinsic activation pathway and is considered a sensitive measure of bleeding [39]. It has long been established that chemically modified heparin derivatives, in which the overall level of sulfation has been reduced, show greatly diminished activities [40-42] and this is confirmed for most of the modified derivatives (**Table 2**), in particular **P3**, which exhibited strongly diminished activity. Less is known concerning the ability of sulfated polysaccharides to activate the extrinsic pathway. Charged polysaccharides are also known to act at the common pathway *via* antithrombin and factor Xa, and this activity has also been measured (**Table 2**). Again, most showed very weak activity, including **P3.**

The binding of viruses to host cells is likely to involve multivalent interactions at several levels. First, there are potentially multiple binding sites on cell surface macromolecules, particularly heparan sulfate or other glycosaminoglycan polysaccharides but, also, the geometry of the viral coat, comprising many identical subunits, provides many possible sites of attachment. Inhibiting this attachment, or reversing it, will require the use of molecules capable of forming multiple bonds, which these anionic polysaccharides are ideally suited to do. Analysis of the H5N1 HA (Influenza A virus A/*Cygnus olor*/Italy/742/2006) amino acid sequence and comparison with the sequences of all of the currently known 437 heparin binding proteins identifies a number of possible heparin binding sequences (HBS) within the receptor binding domain (RBD) of HA (**Figure 3**) [43, 44]. This analysis indicates that heparin inhibits invasion of the H5-pseudotyped-HIV virus by sterically hindering the RBD [45] of HA.

Figure 3. *Exploring the potential location of the binding of heparin derivatives in H5.* The receptor binding domain (RBD) (blue) and predicted heparin binding sequences (HBS) (red) of haemagglutinin originating from Influenza A virus A/*Cygnus olor*/Italy/742/2006. The amino acid sequence numbering included the signal peptide, at the beginning of the protein. The RBD of H5N1 HA is comprised of three stretches of amino acid stretches 143-147 and 228-237 that are present in loops and amino acids 194-204 which are in a helical region. Furthermore, amino acids W162, H192 and Y204 are also known to form part of the RBD [45].

The HBSs within HA were predicted by examining the basic amino acid sequences in HA and comparing those to the basic sequence found in the 437 current catalogued heparin binding proteins [43, 44]. The analysis involved using the Levenshtein distance to compare the amino acid sequences, with highly conserved sequences being found using network analysis.

4. Conclusions.

Chemically modified heparin derivatives have been shown to inhibit influenza H5N1 invasion using a pseudo-type assay. Reducing sulfation levels resulted in more potent inhibitors of invasion than the parental porcine mucosal heparin, implying that further optimisation is likely to be feasible. Furthermore, the most relevant side-effects associated with heparin administration are severely attenuated, making modified heparins attractive possibilities for further development.

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Notes and references

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Electronic Supplementary Information (ESI) available: S1. Chemical modifications of heparin. S2. Preparation of modified heparins. S3. Chemically modified heparin polysaccharide purity. S4. NMR characterisation of chemically modified heparin polysaccharides. S5. Heparin derivatives do not neutralise the VSVg-pseudotyped lentiviral vectors. See DOI: 10.1039/b000000x/

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