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Molecular Wipes: Application to Epidemic Keratoconjuctivitis

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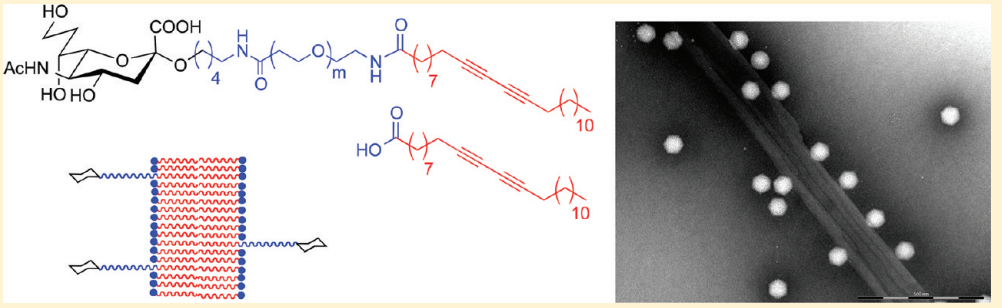
Supporting Information

ABSTRACT:



Epidemic keratoconjunctivitis (EKC) is a severe disease of the eye, caused by members of the Adenoviridae (Ad) family, with symptoms such as keratitis, conjunctivitis, pain, edema, and reduced vision that may last for months or years. There are no vaccines or antiviral drugs available to prevent or treat EKC. It was found previously that EKC-causing Ads use sialic acid as a cellular receptor and demonstrated that soluble, sialic acid-containing molecules can prevent infection. In this study, multivalent sialic acid constructs based on 10,12-pentacosadiynoic acid (PDA) have been synthesized, and these constructs are shown to be efficient inhibitors of Ad binding (IC_{50} = 0.9 μM) and Ad infectivity (IC_{50} = 0.7 μM). The mechanism of action is to aggregate virus particles and thereby prevent them from binding to ocular cells. Such formulations may be used for topical treatment of adenovirus-caused EKC.

INTRODUCTION

The adenovirus family contains more than 50 types known to cause disease in humans.[1] Historically, adenovirus type 8 (Ad8), Ad19, and Ad37 are the main causative agents of epidemic keratoconjunctivitis (EKC), which is a severe infection of the eye, causing symptoms such as keratitis, conjunctivitis, pain, lacrimation, and reduced vision that may last for months or even years.[2,3] At present, there are no licensed antiviral agents for treatment of this disease. The viruses are transferred by contact, such as handshakes, door knobs, towels, or contaminated tonometers at ophthalmic departments.[4] Consequently, EKC is more common in densely populated areas, predominantly in Asia but also in North America and Europe.[5−7] In Japan, for example, up to a million individuals fall ill with EKC every year.[8] Patients are usually advised to stay home from work or school, resulting in substantial economic losses.[9]

We have previously demonstrated that EKC-causing Ads bind via the knob domain of the protruding, homotrimeric fiber protein to sialic acid-containing cell surface molecules and use these as functional receptors for infection of ocular cells in vitro.[10−13] The sialic acid-containing glycans resemble, or are identical to, the branched hexasaccharide present in the GD1a ganglioside, in which the two branches terminate with sialic acids. However, the receptor used by Ad37 is not the GD1a ganglioside itself, but rather glycoproteins that contain the GD1a glycan, or very similar glycan motifs. Interestingly, molecular modeling, X-ray crystallography, and NMR show that the two terminal sialic acids of GD1a bind to two of the three sialic acid binding sites in the trimeric Ad37 knob.[14] It is reasonable to assume that the interaction between Ad37 and ocular cells is an example of a multivalent interaction, similar to the interactions between influenza virus and bronchial epithelial cells. However, one main difference between Ad37−GD1a interaction and the interactions between sialic acid and influenza hemagglutinin is that the carbohydrate binding sites lie around the central cavity of the fiber knob, rather than at the tip, as in the influenza hemagglutinin.[13,14] Multivalent interactions are characterized by the simultaneous binding of several receptors from, for example, the virus and several ligands at the cell surface. Despite the low binding constant of each individual ligand−receptor interaction, the combined multivalent interaction may be very strong.[15]

In earlier efforts, multivalent, albumin-based, sialylated compounds have been shown to efficiently bind to and aggregate Ad37 virions and, thus, prevent these from binding to and infecting human ocular cells.[16,17] A 13-valent sialic acid−human serum albumin conjugate was thus shown to be 1000 times more efficient than sialic acid monosaccharides, on a molar basis,

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in preventing Ad37 from binding and infecting human corneal epithelial (HCE) cells. On the basis of these findings we hypothesized that other multivalent sialic acid conjugates may function as antiviral drugs for topical treatment of EKC.

Multivalent sialic acid conjugates have been used earlier for the binding of influenza viruses.[15,18] For example, dendrimers carrying sialic acid were found to enhance virus binding 100 times, compared to monovalent sialic acid, and polyacrylamide-based sialic acid conjugates bound to viruses $>10^6$ times stronger than the monovalent sialic acid. However, from a pharmaceutical perspective, liposomes are more attractive carriers of sialic acid. The sialic acid may be coupled to a lipid tail, thus forming a single entity rather than an unstructured polymer, and incorporated in the liposomes. Furthermore, the concentration of sialic acid can easily be varied in such liposomal preparations. Sialic acid-containing, lipid-based systems have been shown to enhance binding to influenza viruses by a factor of 10^4-10^6.[15] In addition, the high mobility of the sialic acid units in the membrane may also facilitate optimized binding to the virus.

Liposomes can be formed from a large variety of lipids, but to design artificial membranes, 1,3-diacetylenic acids, such as 5,7-docosadiynoic acid and 10,12-pentacosadiynoic acid (PDA), are attractive because the formed liposomes can be polymerized by a 1,4-addition reaction induced by ultraviolet radiation.[19] Interestingly, these polydiacetylene polymers can undergo distinct chromatic transitions when they bind to, for example, viruses or neurotoxins, and they have thus found wide applications as sensors.[20]

It is known that some 1,3-diacetylenic acid liposomes form large multilamellar extended sheets when cooled to 4 °C, irrespective of the size of the preformed vesicles (i.e., 50, 100, or 300 nm).[21,22] Such multilamellar sheets would simulate a cell surface and could therefore be suitable for binding of viruses and also for ocular treatment.

The aims of this investigation were to design sialic acid-containing multivalent lipid-based species based on the PDA system and evaluate these in biological in vitro systems to find candidate drugs for the treatment of EKC. However, because we want the sialic acids to move freely in the membrane, we preferred not to polymerize the 1,3-diacetylenic acids. In addition, because human adenoviruses (unlike influenza A viruses) do not have neuraminidases, which cleave *O*-coupled sialic acids, we chose to use this approach (*O*-coupling) rather than the corresponding *C*-sialosides, which have been used in earlier approaches to influenza treatment.[20,23,24]

RESULTS AND DISCUSSION

Our sialic acid constructs were composed of three different regions, that is, the sialic acid unit, a polar linker to separate the sialic acid from the tail, and a nonpolar tail to be embedded in the PDA sheets or liposomes (Figure 1).

Five compounds, **6**, **7**, and **8a**−**8c**, were synthesized using a block synthesis (Scheme 1). Thus, sialic acid coupled with an 9-fluorenylmethoxycarbonyl (Fmoc)-protected aminopentanol (**1**[17]) was deprotected using piperidine in DMF. The crude product was coupled, using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl), with Fmoc-protected polyethylene glycol linkers of different lengths (i.e., Fmoc−NH−CH_2CH_2O−PEG_n−CH_2CH_2−COOH, n = 2, 4, or 11) to get **2a**−**2c**. Compounds **2a**−**2c** were then deprotected using piperidine in DMF, and the crude products were coupled,

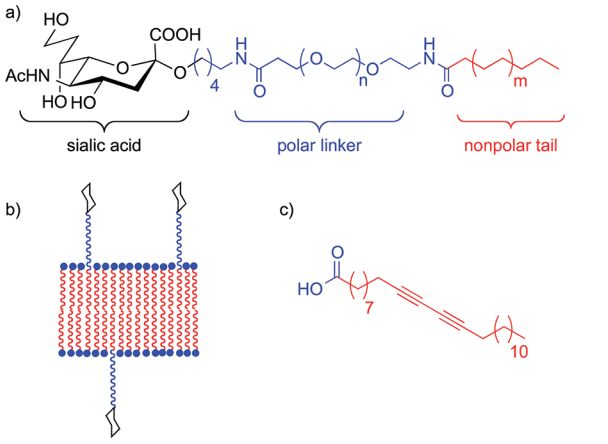

Figure 1. (a) General design of sialic acid conjugates. The length of the polar linker is varied as well as the structure of the nonpolar tail. (b) Sheets formed by PDA and the sialic acid conjugates. (c) Structure of PDA.

using EDC·HCl, with stearic acid (to get compound **3**), with tetracosanoic acid (**4**), or with PDA (**5a**−**5c**). (See the Supporting Information for experimental data and spectra of compounds **2**−**5**.) Compounds **3**−**5** were then deprotected by treatment with NaOMe (0.05 M in MeOH) for 5 h, followed by the addition of 5 equiv of NaOH and continued stirring for another 24 h. The final compounds (**6**−**8**) were purified by column chromatography on SiO_2 using CH_2Cl_2/MeOH/H_2O systems.

Compounds **6**−**8** and PDA were mixed in CH_2Cl_2, the solvent was evaporated, and 10 mL of water was added; the mixtures were probe sonicated until a clear solution was obtained (approximately 15 min) or extruded. The IC_{50} values for the different formulations were investigated in a binding assay using ^{35}S-labeled Ad37 virions and HCE cells. The results of such an experiment are shown in Figure 2.

The effect of different lipid tails was investigated using stearic acid (STA), tetracosanoic acid (TA), and PDA. Thus, compounds **6**, **7**, and **8b**, that is, sialic acid coupled via a polyethylene glycol linker to the three different acids, were mixed with PDA in a 1:9 ratio, and the mixtures were extruded to give, initially, 100 nm liposomes with a total concentration of 1 mM (Table 1, entries 1, 2, and 4). Whereas both TA and STA resulted in high IC_{50} values (>20 μM with respect to sialic acid), the PDA conjugate resulted in a much lower IC_{50} value (4 μM).

We next hypothesized that the linker length is important for optimal virion interaction and that too short or too long linkers may be detrimental to the binding. To investigate the effect of the linker length, compounds **8a**−**8c** were mixed with PDA (1:9 ratio) and extruded to 100 nm liposomes (Table 1, entries 3−5). Thus, the compound equipped with the shortest linker (**8a**) resulted in an IC_{50} value >50 μM, whereas the compounds carrying longer linkers (**8b** and **8c**) resulted in lower IC_{50} values (4 and 7 μM, respectively). The PEG_4 linker was chosen for further experiments.

To investigate the effect of the formulation techniques, mulivalent lipid-based species were formed from **8b** and PDA (1:9 ratio) by the extrusion method (method A) and by probe sonication (method B) (Table 1, entries 3 and 6). The formulation prepared with method B inhibited virus binding at slightly lower concentrations (IC_{50} = 0.9 μM) compared to the formulations prepared with method A (IC_{50} = 4 μM). Probe sonication

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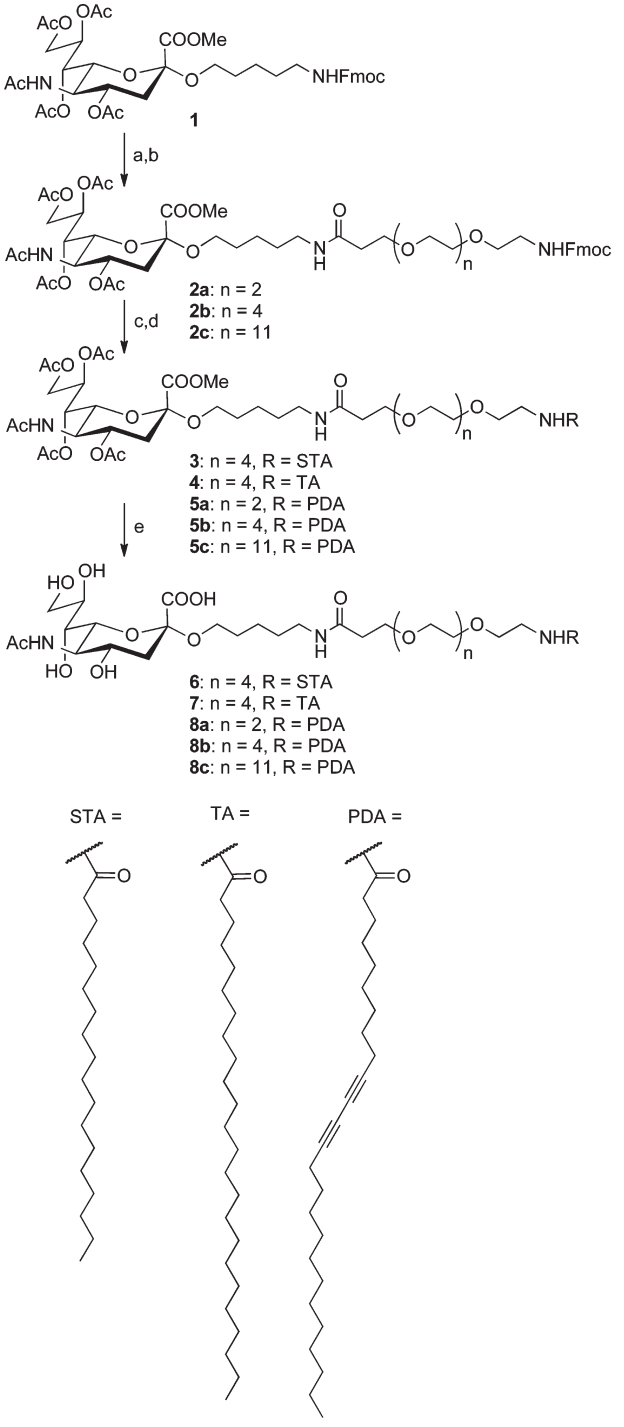
Scheme 1. Synthesis of Sialic Acid Conjugates



a, piperidine, DMF, 2 h. b, Fmoc−NH−CH_2CH_2O−PEG_n−CH_2CH_2−COOH, EDC·HCl, CH_2Cl_2, 18 h. c, piperidine, DMF, 3 h. d, fatty acid, EDC·HCl, Et_3N, CH_2Cl_2, 17 h. e, NaOMe, MeOH, NaOH, H_2O, 29 h.

usually forms liposomes with a size of 50 nm, whereas the extrusion technique, using a filter size of 100 nm, results in liposomes of 100 nm. In both methods A and B, the liposomes were cooled to 4 °C to enable formation of sheets.[21,22] The slight difference in activities of sheets prepared according to the two methods indicated that the sheets were similar.

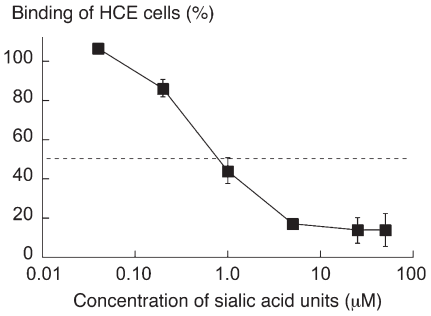


Figure 2. Effect of compound **8b** (Table 1, entry 6) on Ad37 binding to HCE cells. The inhibitory effect of increasing concentrations of **8b** (10 mol % in PDA liposomes) is shown as percent binding of ^{35}S-labeled Ad37 virions to HCE cells. Virus binding was quantified with a scintillation counter. The results are presented as the mean ± SD of duplicate samples from two independent experiments.

Table 1. IC_{50} Binding Values for Mulivalent Lipid-Based Species[a]

entry	compound	tail	linker	formulation method[b]	ratio[c]	IC_{50} (μM)
1	**6**	STA	PEG4	A	1:9	>50
2	**7**	TA	PEG4	A	1:9	21
3	**8a**	PDA	PEG2	A	1:9	>50
4	**8b**	PDA	PEG4	A	1:9	4
5	**8c**	PDA	PEG11	A	1:9	7
6	**8b**	PDA	PEG4	B	1:9	0.9
7	**8b**	PDA	PEG4	B	0.1:9.9	>5
8	**8b**	PDA	PEG4	B	0.5:9.5	<25
9	**8b**	PDA	PEG4	A	2:8	26

[a] All preparations are 1 mM in water with respect to total content of PDA and compounds **6**−**8**. [b] "A" indicates that the formulations were prepared with the extrusion method, and "B" indicates preparation with probe sonication. [c] Ratio of compounds **6**−**8** to PDA.

Finally, to investigate the effect of the concentration of sialic acid on the surface of the preparations, multivalent lipid-based species were produced from PDA and **8b** by probe sonication, using various amounts of **8b** (Table 1, entries 6−9). Thus, a ratio of 1:9 gave an optimized binding, and both lower and higher ratios resulted in significantly higher IC_{50} values. It has been shown before, using a series of *C*-sialosides coupled to PDA, that the best binding to influenza viruses appeared at a mole percentage of sialoside at the liposome surface of 5%, whereas higher and lower concentrations reduced the efficiency.[24] It is reasonable to assume that if the overall density of sialic acid in a formulation becomes too high, the sialic acid-binding fiber knob proteins are prevented by steric hindrance from interacting efficiently.

The inhibitory effect of the formulations was confirmed in infectivity experiments, in which Ad37 virions were preincubated with various concentrations of liposomal preparations of compound **8b** (Table 1, entry 6) and allowed to infect HCE cells. As expected, this formulation prevented infection efficiently, and 0.7 μM was sufficient to inhibit 50% of the infection (Figure 3). Thus, we conclude that these formulations are efficient inhibitors of Ad37 infection of HCE cells.

To confirm that the inhibitory effect was due to aggregation of Ad37 virions, which prevent the virions from binding to HCE cells, we performed a newly developed aggregation assay.[17]

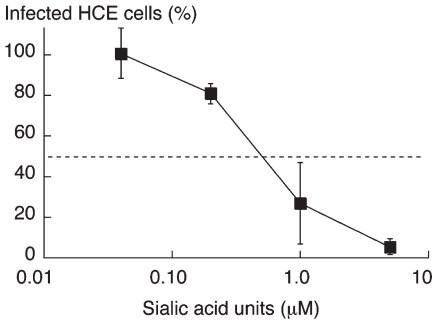


Figure 3. Effect of mulivalent lipid-based species of **8b** on Ad37 infection of HCE cells. The inhibitory effect of increasing concentrations of the formulation is shown as percent infection of HCE cells by Ad37 virions. Virus infection was quantified with immunofluorescence microscope. The results are presented as the mean ± SD of duplicate samples from three independent experiments.

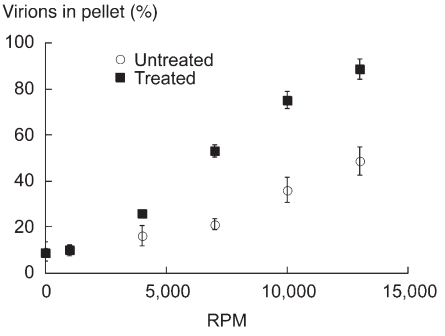


Figure 4. Ad37 virions are aggregated in the presence of mulivalent lipid-based species of **8b** (Table 1, entry 4). The aggregating effect is shown as the percentage of radioactively labeled Ad37 virions in the pellet (the 10 μL in the bottom of the test tube) compared to the total amount of radioactivity in the pellet and the supernatant (top 90 μL) after incubations with or without sialic acid-containing formulations and subsequent centrifugation at different speeds.

After preincubation of formulation **8b** (Table 1, entry 4) with ^{35}S-labeled Ad37 virions, the samples were centrifuged at different revolutions per minute (rpm) to separate aggregated virions (ending up in the pellet, which corresponds to the 10 μL in the bottom of the test tube) from nonaggregated virions (staying in the supernatant, which corresponds to 90 μL in the top of the test tube). At no or low (1000 rpm) speed, most virions remained in the supernatant, and only low levels were found in the pellet, irrespective of whether the virions were preincubated with or without the mulivalent lipid-based species (Figure 4). However, at increasing speed more virions ended up in the pellet, and fewer virions remained in the supernatant. As expected, the amount of virions ending up in the pellet was much more pronounced if the virions had been preincubated with the mulivalent lipid-based species, as compared to the control. These results demonstrate that sialic acid-containing formulations are capable of aggregating Ad37 virions.

As mentioned before, PDA is known to form sheetlike structures.[21,22] In agreement with this, negative stain transmission electron microscopy (nsTEM) images of the multivalent lipid-based species showed heterogeneous, sheetlike structures with a large variation in size and morphology.

Upon incubation of the formed sheets with adenoviruses at 37 °C, we observed a localization of the viruses close to the vicinity of the multilamellar sheets (Figure 5). It seems reasonable that the viruses bind to the sialic acid in a multivalent fashion and thus stick to the sheets. The process was very fast, and we did not observe any differences over time, up to 4 h of incubation.

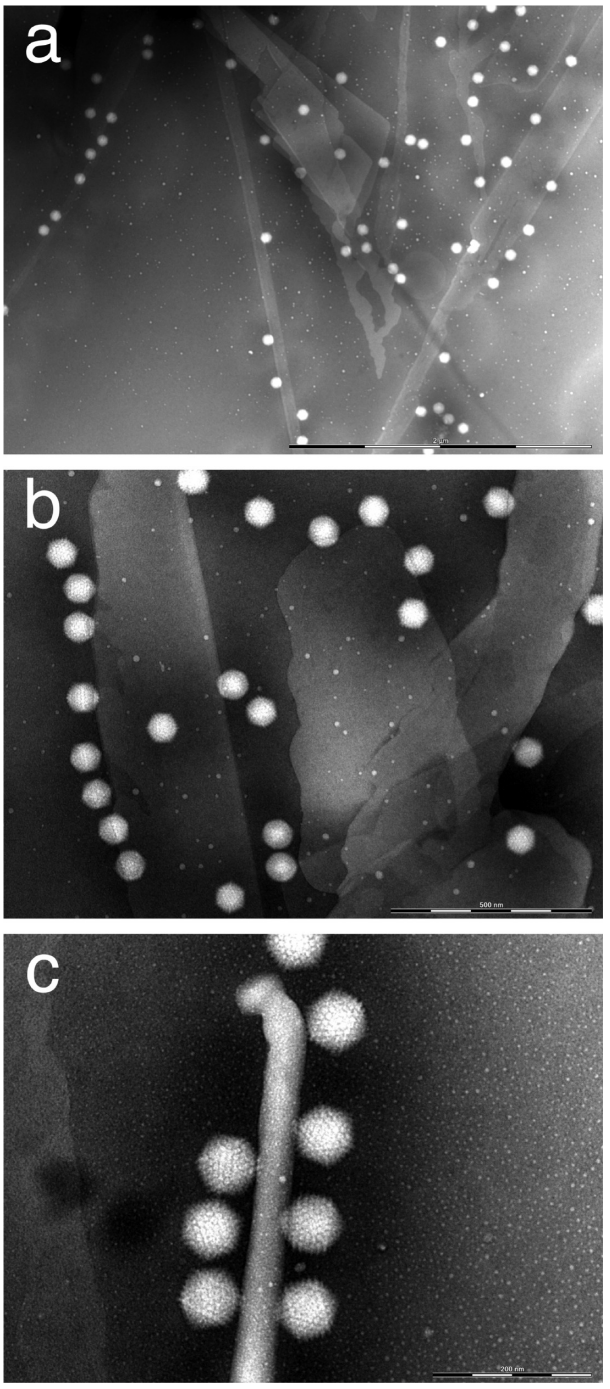


Figure 5. nsTEM images of Ad37 virions mixed with sheets of compound **8b** in 0.9% NaCl. (a) Scale bar = 2000 nm. (b) Scale bar = 500 nm. (c) Scale bar = 200 nm.

CONCLUSIONS

We have synthesized sialic acid conjugates in which the sialic acid is bound, via a polar PEG linker, to a nonpolar tail. We have optimized both the linker length and the tail and used the

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conjugates to form multilamellar sheets, which simulate a eukaryotic cell surface. These artificial cell surfaces were used to prevent Ad37 virions from binding to and infecting ocular cells. We have thus created "molecular wipes" that may be used to treat epidemic keratoconjunctivitis, a severe infection of the eye, for which there are no approved antiviral drugs.

EXPERIMENTAL SECTION

NMR spectra were recorded with a Bruker Avance II operating at 294 K. ^{1}H NMR spectra were assigned using COSY (2D homonuclear shift correlation) with a gradient selection. Chemical shifts are given in parts per million (ppm) downfield from Me_4Si, with reference to residual $CHCl_3$ (7.26) or MeOH-d_3 (3.31). Mass spectra were recorded on Micromass Q-Tof micro. The purchased reagents were used without further purification. The purities of all tested compounds were determined by a combination of thin layer chromatography and NMR. The purities were found to be >95%.

6,25-Diaza-10,13,16,19,22-pentaoxa-7,26-dioxotritetrakontanyl-5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosylonic Acid (6). **3** (13.6 mg, 0.012 mmol) was dissolved in NaOMe (1.2 mL, 0.05 M, in MeOH). The reaction was stirred for 5 h, and then NaOH (0.12 mL, 1 M, in water) was added to the reaction mixture. The reaction mixture was stirred for 24 h and was then quenched with Duolite C436 until neutral pH was reached. The reaction mixture was filtered, rinsed with MeOH, and concentrated under reduced pressure. The crude was subjected to flash column chromatography (DCM/MeOH/H_2O 80:15:5) to give **6** (3.1 mg, 27%): ^{1}H NMR (400 MHz, MeOD), δ 0.90 (t, 3H), 1.29−1.42 (m), 1.48−1.62 (m), 2.00 (s, 3H), 2.19 (t, 2H), 2.45 (t, 2H), 2.81−2.85 (m, 1H), 3.18 (t, 2H), 3.35−3.38 (m), 3.45−3.89 (m). HRMS calcd for $C_{47}H_{89}N_3O_{16}$ $(M + Na)^+$, 974.6141; found, 974.6130.

6,25-Diaza-10,13,16,19,22-pentaoxa-7,26-dioxononatetrakontanyl-5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosylonic Acid (7). Compound **7** was synthesized in an analogous method to compound **6**, using **4**: yield, 14.8 mg (95%); ^{1}H NMR (400 MHz, MeOD), δ 0.90 (t), 1.29−1.60 (m), 2.01 (s, 3H), 2.19 (t, 2H), 2.45 (t, 2H), 2.81−2.84 (m, 1H), 3.12−3.19 (m), 3.35−3.37 (m), 3.47−3.87 (m). HRMS calcd for $C_{53}H_{101}N_3O_{16}$ $(M + Na)^+$, 1058.7080; found, 1058.6575.

6,19-Diaza-10,13,16-trioxa-7,20-dioxo-29,31-tetratetrakontadiynyl-5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosylonic Acid (8a). Compound **8a** was synthesized in an analogous method to compound **6**, using **5a**: yield, 22.4 mg (73%); ^{1}H NMR (400 MHz, MeOD), δ 0.90 (m), 1.30−1.40 (m), 1.47−1.63 (m), 2.01 (s, 3H), 2.18−2.26 (m), 2.45 (t, 2H), 2.81−2.85 (m, 1H), 3.17 (t, 2H), 3.36 (t, 2H), 4.45−3.88 (m). HRMS calcd for $C_{50}H_{87}N_3O_{14}$ $(M + Na)^+$, 976.6086; found, 976.5989.

6,25-Diaza-10,13,16,19,22-pentaoxa-7,26-dioxo-35,37-pentakontadiynyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosylonic Acid (8b). Compound **8b** was synthesized in an analogous method to compound **6**, using **5b**: yield, 16.6 mg (84%); ^{1}H NMR (400 MHz, MeOD), δ 0.90 (t, 3H), 1.27−1.40 (m), 1.47−1.63 (m), 2.01 (s, 3H), 2.12−2.26 (m), 2.45−2.48 (m, 2H), 2.81−2.85 (m, 1H), 3.10−3.23 (m, 2H), 3.35−3.38 (m, 2H), 3.45−3.88 (m). HRMS calcd for $C_{54}H_{95}N_3O_{16}$ $(M + Na)^+$, 1064.6610; found, 1064.6732.

6,46-Diaza-10,13,16,19,22,25,28,31,34,37,40,43,46-dodecaoxa-7,47-dioxo-56,58-henheptakontadiynyl-5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosylonic Acid (8c). Compound **8c** was synthesized in an analogous method to compound **6**, using **5c**: yield, 17.2 mg (98%); ^{1}H NMR (400 MHz, MeOD), δ 0.90 (t, 3H), 1.30−1.40 (m), 1.49−1.63 (m), 2.01 (s, 3H), 2.18−2.26 (m), 2.46 (t, 2H), 2.81−2.85 (m, 1H), 3.18 (t, 2H), 3.35−3.38 (m, 2H), 3.45−3.88 (m). HRMS calcd for $C_{68}H_{123}N_3O_{23}$ $(M + Na)^+$, 1372.8445; found, 1372.8213.

Liposomal Preparations. Formulations were carried out either by a probe type sonicator (50% output, microtip) or by an extrusion device manufactured by Northern Lipids (Vancouver, BC, Canada) using polycarbonate membrane filters, pore size = 100 nm.

Method A (Probe Sonication). PDA (3.4 mg, 9.0 μmol) and compound **8b** (1.0 mg, 1.0 μmol) were mixed in CH_2Cl_2 and put in a glass vial. The solvent was evaporated, and distilled water (10 mL) was added. The mixture was probe sonicated until a clear solution was obtained, approximately 15 min. The warm solution was filtered through a nylon filter (0.8 μm) to remove undispersed lipid and traces of titanium particles from the probe.

Method B (Extrusion). Compound **8b** (1.0 mg, 1.0 μmol) and PDA (3.4 mg, 9.0 μmol) were mixed in $CHCl_3$ and put in a glass vial. The mixture was vortexed, and the solvent was evaporated with nitrogen to yield a thin film of the lipids on the glass. Milli-Q water (10 mL) was added, and the mixture was hydrated at 65 °C under stirring overnight. The liposomal dispersion was then sequentially (10 cycles) passed through a polycarbonate membrane filter (100 nm pore size) above the T_m (65 °C) by an extrusion device.

Binding Assay. The binding assay was performed as described previously.[17] Briefly, purified ^{35}S-labeled Ad37 virions (strain 1477) were incubated with various concentrations of the formulations at 4 °C for 1 h and subsequently incubated for another hour with HCE cells in suspension at 4 °C. Nonbound virions were washed away, and the cell associated radioactivity (bound virions) was quantified with a scintillation counter. All experiments were performed at least three times, unless otherwise indicated in the figure captions, with duplicate samples in each experiment.

Infection Assay. The infection assay was performed as described previously.[17] Briefly, Ad37 virions were incubated with various concentrations of formulations of compound **8b** at 4 °C for 1 h and subsequently incubated for another hour with adherent HCE cells at 4 °C. Nonbound virions were washed away, and the cells were warmed to 37 °C to allow synchronized entry of virons into the cells and subsequent production of viral antigens. After 44 h of incubation at 37 °C, the cells were washed, fixed with methanol, and stained with rabbit anti-Ad37 virion serum and FITC-labeld swine anti-rabbit IgG antibodies. The level of infectivity was then quantified using an immunofluorescence microscope and ImageJ software.[25] All experiments were performed at least three times with duplicate samples in each experiment.

Aggregation Assay. The aggregation assay was also performed as described previously.[17] Briefly, 100 μL of binding buffer (control; DMEM, penicillin−streptomycin, and HEPES at pH 7.4) or 100 μL of the formulation of compound **8b** diluted with binding buffer (test) was added to a V-shaped well containing a small volume of a suspension with 5×10^8 ^{35}S-labeled Ad37 virions. The mixture was kept at 4 °C for 1 h to establish equilibrium between unbound virions and virions bound to the formulation. After incubation, the mixture was centrifuged at different speeds (1000, 4000, 7000, 10000, and 13000 rpm) using a tabletop centrifuge. The radioactivity in the supernatant (top 90 μL; defined as nonaggregated virions) and in the pellet (remaining 10 μL; defined as aggregated virions) was measured using a liquid scintillation counter. All experiments were performed at least three times with duplicate samples in each experiment.

ASSOCIATED CONTENT

Supporting Information. Experimental data for compounds **2**−**5** and ^{1}H NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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ABBREVIATIONS USED

Ad, adenovirus; EDC·HCl, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; EKC, epidemic keratoconjunctivitis; Fmoc, 9-fluorenylmethoxycarbonyl; HCE, human corneal epithelial; PDA, 10,12-pentacosadiynoic acid; STA, stearic acid; TA, tetracosanoic acid

REFERENCES

(1) Benkö, M.; Harrach, B.; Both, G. W.; Russell, W. C.; Adair, B. M.; Adam, E.; de Jong, J. C.; Hess, M.; Johnson, M.; Kajon, A.; Kidd, A. H.; Lehmkul, H. D.; Li, Q.-G.; Mautner, V.; Pring-Åkerblom, P.; Wadell, G. Family Adenoviridae. In *Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses*; Fauquet, C. M., Mayo, M. A., Maniloff, J., Desselberger, U., Ball, L. A., Eds.; Elsevier Academic Press: San Diego, CA, 2005; pp 213−228.

(2) Ford, E.; Nelson, K. E.; Warren, D. Epidemiology of epidemic keratoconjunctivitis. *Epidemiol. Rev.* **1987**, *9*, 244–261.

(3) Gordon, Y. J.; Aoki, K.; Kinchington, P. R. Adenovirus keratoconjunctivitis. In *Ocular Infection and Immunity*; Pepose, J. S., Holland, G. N., Wilhelmus, K. R., Eds.; Mosby: St. Louis, MO, 1996; pp 877−894.

(4) Azar, M. J.; Dhaliwal, D. K.; Bower, K. S.; Kowalski, R. P.; Gordon, Y. J. Possible consequences of shaking hands with your patients with epidemic keratoconjunctivitis. *Am. J. Ophthalmol.* **1996**, *121*, 711–712.

(5) Aoki, K.; Kawana, R.; Matsumoto, I.; Wadell, G.; de Jong, J. C. Viral conjunctivitis with special reference to adenovirus type 37 and enterovirus 70 infection. *Jpn. J. Ophthalmol.* **1986**, *30*, 158–164.

(6) Gottsch, J. D. Surveillance and control of epidemic keratoconjunctivitis. *Trans. Am. Ophthalmol. Soc.* **1996**, *94*, 539–87.

(7) Mueller, A. J.; Klauss, V. Main sources of infection in 145 cases of epidemic keratoconjunctivities. *Ger. J. Ophtalmol.* **1993**, *2*, 224–227.

(8) Kaneko, H.; Suzutani, T.; Aoki, K.; Kitaichi, N.; Ishida, S.; Ishiko, H.; Ohashi, T.; Okamoto, S.; Nakagawa, H.; Hinokuma, R.; Asato, Y.; Oniki, S; Hashimoto, T.; Iida, T.; Ohno, S. Epidemiological and virological features of epidemic keratoconjunctivitis due to new human adenovirus type 54 in Japan. *Br. J. Ophthalmol.* **2011**, *95*, 32–36.

(9) Gordon, Y. J. The evolution of antiviral therapy for external ocular viral infections over twenty-five years. *Cornea* **2000**, *19*, 673–680.

(10) Arnberg, N. Adenovirus receptors, implications for tropism, treatment and targeting. *Rev. Med. Virol.* **2009**, *19*, 165–178.

(11) Arnberg, N.; Edlund, K.; Kidd, A. H.; Wadell, G. Adenovirus type 37 uses sialic acid as a cellular receptor. *J. Virol.* **2000**, *74*, 42–48.

(12) Arnberg, N.; Kidd, A. H.; Edlund, K.; Olfat, F.; Wadell, G. Initial interactions of subgenus D adenoviruses with A549 cellular receptors: sialic acid versus alpha(v) integrins. *J. Virol.* **2000**, *74*, 7691–7693.

(13) Burmeister, W. P.; Guilligay, D.; Cusack, S.; Wadell, G.; Arnberg, N. Crystal structure of species D adenovirus fiber knobs and their sialic acid binding sites. *J. Virol.* **2004**, *78*, 7727–7736.

(14) Nilsson, E. N.; Storm, R. J.; Bauer, J.; Johansson, S. M. C.; Lookene, A.; Ångström, J.; Hedenström, M.; Eriksson, T. L.; Frängsmyr, L.; Rinaldi, S.; Willison, H. J.; Pedrosa Domellöf, F.; Stehle, T.; Arnberg, N. The GD1a glycan is a cellular receptor for adenoviruses causing epidemic keratoconjunctivitis. *Nat. Med.* **2011**, *17*, 105–109.

(15) Mammen, M.; Choi, S.-K.; Whitesides, G. Polyvalent interactions in biological systems: implications for design and use of multivalent ligands and inhibitors. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754–2794.

(16) Johansson, S. M.; Arnberg, N.; Elofsson, M.; Wadell, G.; Kihlberg, J. Multivalent HSA conjugates of 3′-sialyllactose are potent inhibitors of adenoviral cell attachment and infection. *Chembiochem* **2005**, *6*, 358–364.

(17) Johansson, S. M.; Nilsson, E. C.; Elofsson, M.; Ahlskog, N.; Kihlberg, J.; Arnberg, N. Multivalent sialic acid conjugates inhibit adenovirus type 37 from binding to and infecting human corneal epithelial cells. *Antiviral Res.* **2007**, *73*, 92–100.

(18) Sun, X.-L. Recent anti-influenza strategies in multivalent sialyloligosaccharides and sialylmimetic approaches. *Curr. Med. Chem.* **2007**, *14*, 2304–2313.

(19) Tieke, B.; Graf, H.-J.; Wegner, G.; Naegele, B.; Ringsdorf, H.; Benerjie, A.; Day, D.; Lando, J. B. Polymerization of mono- and multilayer forming diacetylenes. *Colloid Polym. Sci.* **1977**, *255*, 521–531.

(20) Charych, D. C.; Nagy, J. O.; Spevak, W.; Bednarski, M. D. Direct colorimetric detection of a receptor-ligand interaction by a polymerized bilayer assembly. *Science* **1993**, *261*, 585–588.

(21) Peek, B. M.; Callahan, J. H.; Namboodiri, K.; Singh, A.; Gaber, B. P. Effect of vesicle size on polymerization of a diacetylene lipid. *Macromolecules* **1994**, *27*, 292–297.

(22) Guo, C. X.; Boullanger, P.; Liu, T.; Jiang, L. Size effect of polydiacetylene vesicles funtionalized with glyoclipids on their colorimetric detection ability. *J. Phys. Chem. B* **2005**, *109*, 18765–18771.

(23) Reichert, A.; Nagy, J. O.; Spevak, W.; Charych, D. Polydiacetylene liposomes functionalized with sialic acid bind and colorimetrically detect influenza virus. *J. Am. Chem. Soc.* **1995**, *117*, 829–830.

(24) Spevak, W.; Nagy, J. O.; Charych, D. H.; Schaefer, M. E.; Gilbert, J. H.; Bednarski, M. D. Polymerized liposomes containing *C*-glycosides of sialic acid: potent inhibitors of influenza virus in vitro infectivity. *J. Am. Chem. Soc.* **1993**, *115*, 1146–1147.

(25) Abramoff, M. D.; Magalhaes, P. J.; Ram, S. J. Image processing with ImageJ. *Biophotonics Int.* **2004**, *11*, 36–42.