Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 341–343

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis of a Polymeric 4-*N*-linked Sialoside which Inhibits Influenza Virus Hemagglutinin

Wen-Yang Wu, Betty Jin, Guy Y. Krippner and Keith G. Watson*

Biota Chemistry Laboratory, Chemistry Department, Monash University, Clayton, Victoria 3168, Australia

Received 28 September 1999; accepted 13 December 1999

Abstract—A multiple sialic acid-bearing polymer **7** has been made in which a novel 4-*N*-substituted sialoside **5** has been coupled to polyacrylamide. The conjugate **7** has been found to inhibit the agglutination of influenza virus to red blood cells with HAI inhibition constants of around 10^{-6} M, based on the sialic acid concentration.

The envelope of influenza type A and B viruses consists of a lipid bilayer derived from the host cell and within the lipid membrane there are two glycoproteins, hemagglutinin (HA) and neuraminidase (NA), which protrude out from the viral surface.[1] Influenza virus hemagglutinin (HA) is responsible for the binding of the virus to target cells by recognising receptor molecules bearing α-5-*N*-acetylneuraminic acid (sialic acid) **1** and thereby initiating viral attachment and infection.[2] In contrast, the function of NA is to cleave sialic acid from the ends of glycoprotein chains and NA is therefore thought to be important in allowing the release of viral particles from cell surfaces and facilitating their movement through mucus.[3] It has long been thought that compounds which bind strongly to either HA or NA would be good candidates for the prevention of influenza infection and indeed, recently discovered tight binding inhibitors of NA have shown great promise in clinical trials.[4,5] On the other hand, the search for small molecules that bind to HA has not been so successful,[6] possibly due to the shallow nature of the HA sialic acid binding region.[7] Sialic acid **1**, and derivatives such as α-methyl sialoside, bind only weakly to HA, with dissociation constants in the millimolar range, whereas some sialic acid derivatives bearing large groups at the 2 and 4 positions have dissociation constants in the micromolar range.[8]

The structures of influenza HA complexed with several different 2- or 4-substituted sialic acid derivatives have been determined by X-ray crystallography,[9] and the structure of sialyllactose and other sialyloligosaccharides bound to HA have also been reported recently.[10] In all cases the position of the sialic acid group and the interactions between the protein and the sialic acid are the same. Importantly, the X-ray structures of the complexes show that the 4-OH of sialic acid points away from the binding site into solution and does not have any interactions with the protein. This is consistent with the observation that 4-substituted sialosides can still bind to HA.[7,8]

It is believed that the strong binding of influenza virus to target cells results from the interaction of the cell-surface sialic acid groups with multiple copies of HA on the virus.[11] Based on this concept of a polyvalent interaction, several groups have prepared synthetic polymers bearing multiple sialic acid substituents and found they are highly effective in blocking the attachment of virus to red blood cells (see, for example, refs 12–14). In all of the reported synthetic polymers bearing sialic acid the carbohydrate groups are linked through the 2-position, but in view of the structural information mentioned above we wondered if it might be possible to make multivalent binders of HA by linking sialic acid onto a polymer via the 4-position. We now report that when polyacrylamide is substituted by multiple copies of 4-linked sialic acid groups, it is indeed highly effective in preventing the attachment of influenza virus to red blood cells.

For the attachment of a stable linking group at the 4-position of sialic acid we preferred to start with a 4-amino sialic acid derivative so that reaction with an isocyanate would give a non-hydrolysable urea functionality which should also be suitable for hydrogen

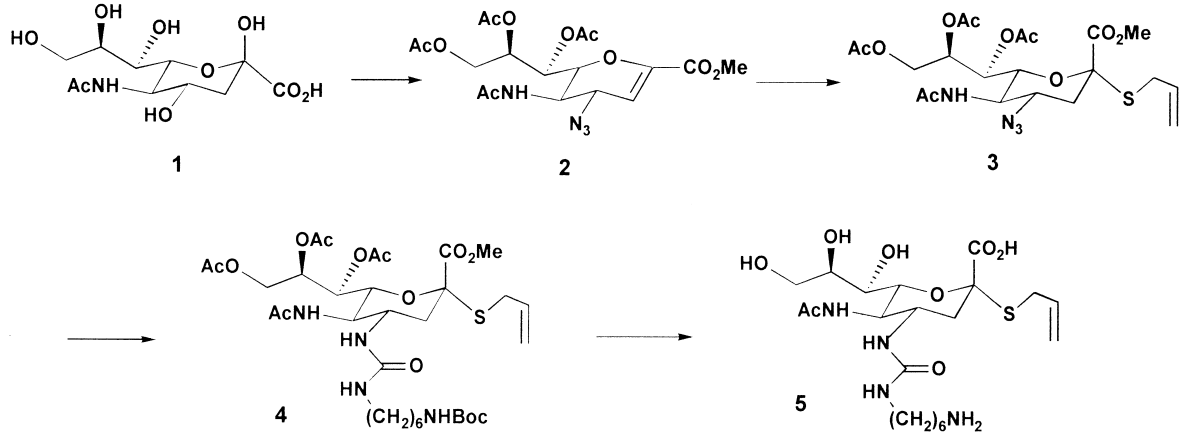
*Corresponding author. Fax: +61-3-9905-5505; e-mail: k.watson@sci.monash.edu.au

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00007-X

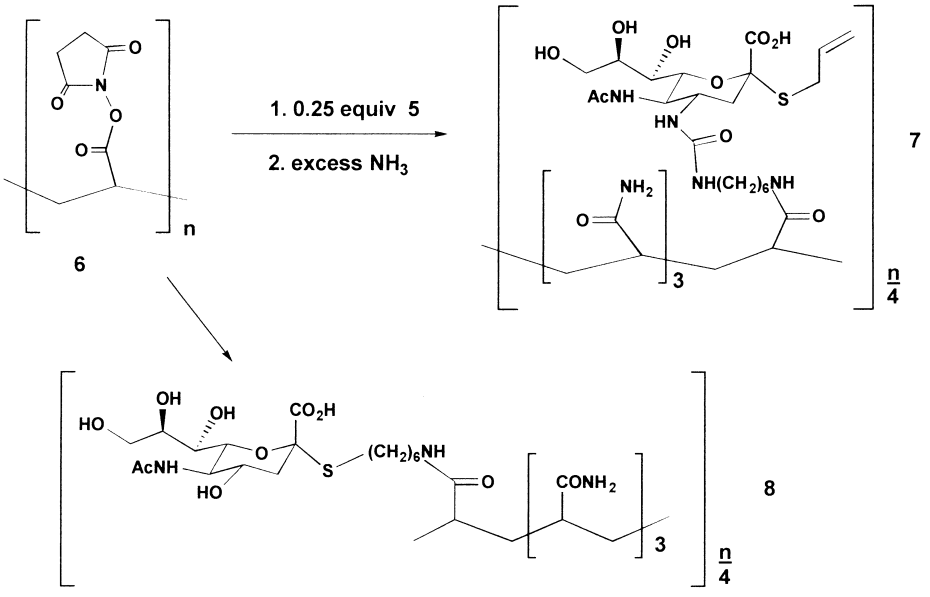
bonding to the nearby serine residue of HA.[9] As outlined in Scheme 1, the known[15] 4-azido-2,3-dehydro sialic acid derivative **2**, was converted into the 4-azido-α-2-thioglycoside **3** via the β-2-chloro[16] and α-2-thioacetate derivatives following the method of Bennett et al.[17] Reduction of the 4-azido group in compound **3** was carried out by hydrogenation on Pd/C to give the 4-amino-α-2-thioglycoside which was reacted with 6-*N*-*t*-Boc-aminohexylisocyanate to provide the protected sialic acid ligand **4**.

Reaction of poly-[*N*-(acryloyloxy)succinimide][18] **6** with 0.25 equiv of the deprotected ligand **5** per activated ester group, followed by quenching of unreacted active ester with aqueous ammonia, gave the polyacrylamide conjugate **7** (Scheme 2). After extensive dialysis to remove any free ligand and then freeze drying of the product, the ^{1}H NMR spectrum of conjugate **7** showed approximately 25% substitution of the polymer backbone with ligand **5**, confirming that complete reaction had occurred. Samples of the same batch of activated ester polymer **6** were also treated directly with ammonia and aqueous sodium hydroxide to give samples of unsubstituted polyacrylamide and polyacrylic acid. By comparison with standard polymers of known molecular weight (MW), and using both viscosity measurements and gel permeation chromatography (GPC) analysis our unsubstituted polymers were found to have a MW of around 50,000. For comparison with the 4-linked sialic acid conjugate **7**, a sample of a 2-linked sialic acid conjugate **8**, similar to those reported by other workers, was also prepared from the same batch of polymer **6** as shown in Scheme 2, and following the general approach described by Mammen et al.[18]

The conjugates **7** and **8** were tested for the inhibition of agglutination of chicken erythrocytes caused by an influenza H3 subtype (X-31) and two H1 subtypes (A/Tokyo and G70C), using a routine HAI assay.[18,19] The results are shown in Table 1, together with results for unsubstituted polyacrylamide of similar MW and also the natural sialylglycoprotein fetuin. The potent inhibitory activity of the polymer **7** indicates that, despite the change in sialic acid attachment point, the



Scheme 1.



Scheme 2.

Table 1. Hemagglutinin inhibition results with various influenza strains

Compound	K_i (μM of sialic acid units)		
	X-31	A/Tokyo	G70C
7	0.97	< 0.48	2.93
8	2.93	3.90	< 0.48
Polyacrylamide[a]	93.75	187.5	187.5
Fetuin	< 0.48	5.86	0.73

[a]For polyacrylamide the MW was assumed to be 280 (equivalent to 4 acrylamide units).

polymer is able to bind to the influenza HA in a polyvalent manner and with roughly equal affinity to the 2-linked conjugate **8**. The activity seen with sialic acid conjugate **8** is consistent with that reported by other workers (e.g. refs 12–14) for similar 2-linked sialic acid conjugates and the results for fetuin and polyacrylamide are also in reasonable agreement with reported values (e.g. refs 12–14 and 18).

To have any potential for clinical effectiveness against influenza it would be important for a compound to show activity on all known influenza strains. The literature suggests that previously reported 2-linked sialic acid polymers are variable in their level of activity on different influenza strains,[20] possibly due to the fact that when the sialic acid groups bind to HA the polymer backbone and linking group are brought close to the HA surface, which varies from strain to strain.[21] Given that linkage through the sialic acid 4-position would hold the polymer backbone further away from the HA surface, it may be that polymeric 4-linked sialic acid conjugates like compound **7** will show less variability in their activity on different influenza subtypes.

Acknowledgements

We thank Dr. Jenny McKimm-Breschkin and Mandy McDonald of the Biomolecular Research Institute in Melbourne for carrying out the influenza hemagglutination assays.

References and Notes

1. Webster, R. G.; Bean, W. J.; Gorman, O. T.; Chambers, T. M.; Kawaoka, Y. *Microbiol. Reviews* **1992**, *56*, 152.
2. Wiley, D. C.; Skehel, J. J. *Ann. Rev. Biochem.* **1987**, *56*, 365.
3. Liu, C.; Eichelberger, M. C.; Compans, R. W.; Air, G. M. *J. Virology* **1995**, *69*, 1099.
4. Silagy, C. A. *The Lancet* **1998**, *352*, 1877.
5. Calfee, D. P.; Hayden, F. G. *Drugs* **1998**, *56*, 537.
6. Hoffman, L. R.; Kuntz, I. D.; White, J. M. *J. Virology* **1997**, *71*, 8808.
7. Sauter, N. K.; Hanson, J. E.; Glick, G. D.; Brown, J. H.; Crowther, R. L.; Park, S.-J.; Skehel, J. J.; Wiley, D. C. *Biochemistry* **1992**, *31*, 9609.
8. Weinhold, E. G.; Knowles, J. R. *J. Am. Chem. Soc.* **1992**, *114*, 9270.
9. Watowich, S. J.; Skehel, J. J.; Wiley, D. C. *Structure* **1994**, *2*, 719.
10. Eisen, M. B.; Sabesan, S.; Skehel, J. J.; Wiley, D. C. *Virology* **1997**, *232*, 19.
11. Pritchett, T. J.; Paulson, J. C. *J. Biol. Chem.* **1989**, *264*, 9850.
12. Reuter, J. D.; Myc, A.; Hayes, M. M.; Gan, Z.; Roy, R.; Qin, D.; Yin, R.; Piehler, L. T.; Esfand, R.; Tomalia, D. A.; Baker, J. R. *Bioconjugate Chem.* **1999**, *10*, 217.
13. Sigal, G. B.; Mammen, M.; Dahmann, G.; Whitesides, G. M. *J. Amer. Chem. Soc.* **1996**, *118*, 3789.
14. Tuzikov, A. B.; Byramova, N. E.; Bovin, N. V.; Gambaryan, A. S.; Matrosovich, M. N. *Antiviral Res.* **1997**, *33*, 129.
15. von Itzstein, M.; Wu, W.-Y.; Jin, B. *Carbohyd. Res.* **1994**, *259*, 301.
16. Sabesan, S.; Neira, S.; Wasserman, Z. *Carbohyd. Res.* **1995**, *267*, 239.
17. Bennett, S.; von Itzstein, M.; Kiefel, M. J. *Carbohyd. Res.* **1994**, *259*, 293.
18. Mammen, M.; Dahmann, G.; Whitesides, G. M. *J. Med. Chem.* **1995**, *38*, 4179.
19. The compounds were serially diluted across 96-well microtitre plates containing PBS. Suspensions of virus, diluted to 4 HA units in PBS were added to the wells and after 2 h at 4 °C a 0.5% suspension of chicken erythrocytes was added to each well. After 1 h at 4 °C the lowest concentration of compound that inhibited agglutination of the erythrocytes was determined visually. All assays were run in duplicate.
20. Itoh, M.; Hetterich, P.; Isecke, R.; Brossmer, R.; Klenk, H.-D. *Virology* **1995**, *212*, 340.
21. Suzuki, Y. *Prog. Lipid Res.* **1994**, *33*, 429.