reactions

MDPI

Article

Thiol-Ene Reaction of Heparin Allyl Ester, Heparin 4-Vinylbenzyl Ester and Enoxaparin

Richard T. Taylor * and Dhruba P. Poudel

Department of Chemistry and Biochemistry, Miami University, Oxford, OH 45056, USA
* Correspondence: taylorrt@miamioh.edu

Abstract: Heparin allyl ester and heparin 4-vinylbenzyl ester were prepared and examined for their potential for thiol-ene reaction using both free radical initiators and photochemistry. While both undergo reaction with mercaptoacetic acid, the allyl ester adduct proved to be somewhat more labile. Several more examples of adducts from heparin 4-vinylbenzyl ester are reported. Similar reactions on enoxaparin, where the reaction site is solely at the non-reducing end of the molecule, are also reported. These reactions may show promise as a strategy in the development of drug conjugates.

Keywords: heparin esters; enoxaparin; thiol-ene reaction

1. Introduction

Polysaccharides and other glycopolymers are attractive candidates for a variety of biomedical applications, including drug delivery, tissue engineering and sensing [1]. The choice of polymer in drug delivery often involves polymers with little inherent biological activity; however, polymers with activity may confer advantages by promoting binding to targeted locations [2].

In this context, heparin is an attractive candidate, given its well-documented binding to antithrombin and to the spike protein of SARS CoV-2 [3]. Our prior experience in other contexts with the thiol-ene click reaction [4] suggested to us that the thiol-ene reaction might be applied to conjugation of heparin derivatives, since it has been commonly used in other bioconjugation reactions [5–7].

Heparin is a complex polyaminoglycan [8]. While heparin molecules may have some structural diversity, there are some general commonalities. The average molecular weight ranges typically around 12,000 to 15,000 g/mol or about 60–70 saccharide units. The saccharide units vary, but feature alternating units of an aminoglycan unit and a carboxylate glycan unit. Stereochemistry along the sequence varies, and, in addition to the carboxylate, sulfate groups arrayed about the hydroxyl and amino sites provide an overall negative charge at physiological pH, rending the polymer water soluble. These features allow heparin to bind to positively charged proteins, most notably thrombin. The amino group can bear an acetyl group, a sulfate or be a free amine. A common disaccharide sequence is shown in Figure 1.

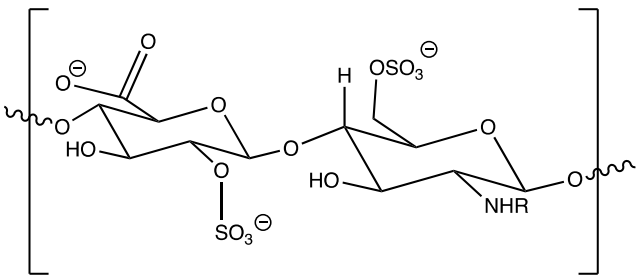

Figure 1. Representative disaccharide units in heparin.

check for updates

Citation: Taylor, R.T.; Poudel, D.P. Thiol-Ene Reaction of Heparin Allyl Ester, Heparin 4-Vinylbenzyl Ester and Enoxaparin. *Reactions* **2022**, *3*, 442–450. https://doi.org/10.3390/reactions3030031

Academic Editor: Dmitry Yu. Murzin

Received: 11 August 2022
Accepted: 29 August 2022
Published: 5 September 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

While there are no alkenyl groups present in heparin, the alkylation of the carboxylate groups can be carried out on the readily available benzalkonium salt of heparin. We reasoned that such ester formation would allow introduction of an appropriate alkenyl group. We report herein on our efforts with two such esters: heparin allyl ester and heparin 4-vinylbenzyl ester.

The large number of carboxylate groups in every heparin chain implies that the thiol-ene conjugation will be highly loaded. Widespread modification may not be optimal in many contexts [9]. While the current work was designed to determine the feasibility of the reaction, a more limited degree of esterification might be required to preserve biological activity.

With the considerations stated above in mind, we also report on thiol-ene reactions of enoxaparin. Enoxaparin is a low molecular weight heparin derivative. It is formed by a base treatment of heparin benzyl ester at a controlled temperature and time. The polysaccharide is cleaved and each fragment bears a single alkenyl group at the non-reducing end of the fragment [10]. The end group saccharide is shown in Figure 2. Enoxaparin includes the bioactive saccharide sequences of heparin, and thus retains much of the biological activity, including thrombin binding and SARS-CoV-2 spike protein binding [11,12]. We reasoned that the terminal alkenyl group would be available for thiol-ene chemistry reactions. It is particularly noteworthy that the reaction would take place at the non-reducing end, since it has been demonstrated that structural features at the reducing end have some consequence for biological activity [13]. Conjugation of enoxaparin at the reducing end has been reported [14], as has pegylation of the carboxylate groups along the enoxaparin chain [15]. Ozonolysis of the non-reducing end of enoxaparin proceeds with no loss of activity [16]. There is one report of the Michael addition of a thiol to enoxaparin esters [17]. However, this addition requires the esterificathetion of enoxaparin (after formation of a benzethonium salt), addition of the thiol and ester hydrolysis. We report on our efforts to carry out such additions with native enoxaparin, obviating the need for the esterification-hydrolysis steps.

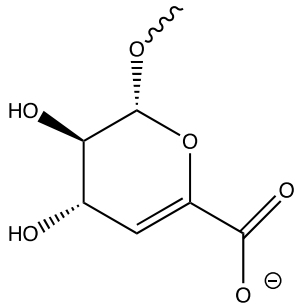

Figure 2. Non-reducing end of enoxaparin.

2. Materials and Methods

2.1. General Information

All reactions were carried out in open air. Commercial reagents and solvents were used as received. Samples of heparin benzalkonium salt and enoxaparin were provided by Smithfield Bioscience. NMR spectra were recorded with a Bruker Advance 500 MHz NMR instrument (Bruker, Billerica, MA, USA) at 298 K in D_2O as solvent. IR spectra were obtained from a Perkin Elmer Spectrum One FT-IR Spectrometer with ATR attachment (Perkin-Elmer, Waltham, MA, USA).

*2.2. Preparation of Heparin Allyl Ester (***1***)*

A quantity of 15 g heparin benzalkonium salt was dissolved in 150 mL N,N-dimethylformamide (DMF). To the solution was added 0.8 mL allyl bromide. The flask was covered and stirred at room temperature for 24 h. The solution was added to 450 mL 10% sodium acetate in methanol. The solid was filtered and dissolved in 5%NaCl, then re-

precipitated by adding to 3× volume of methanol. This drop procedure was then repeated, and 4.55 g of white material was isolated. See Supplementary Materials for IR, HNMR and COSY NMR spectra.

*2.3. Preparation of Heparin 4-Vinylbenzyl Ester (**2**)*

To a solution of 10 g heparin benzalkonium salt in 100 mL DMF was added 7.06 mL (7.63 g, 50 mmol) 4-chloromethylstyrene. The solution was stirred at room temperature for 12 h, then poured onto 300 mL of 10% sodium acetate in methanol. The filtrate was dissolved in a 5% solution of NaCl in water, then reprecipitated by addition to 3× volume methanol. The white solid was air dried to afford 3.7 g of a white solid. While the NMR spectrum will be discussed below, the extent of substitution is clearly less than in the case of the allyl ester, but sufficient to allow examination of the thiol-ene reactions. See Supplementary Materials for IR, HNMR and COSY NMR spectra.

*2.4. Thiol-Ene Reaction of Heparin Allyl Ester with Mercaptoacetic Acid (**3**)*

2.4.1. Using Irradiation

A quantity of 10 mL of an aqueous solution of heparin allyl ester (250 mg) was placed in a small recrystallizing dish and 0.1 mL mercaptoacetic acid was added. During stirring, the solution was irradiated with a wide spectral range lamp (American Ultraviolet Company (Lebanon, IN, USA) model: PC-100S; 120 V, 60 Hz, 5 Amp). After two hours, the solution was reduced to a gum, resuspended in water, and subjected to cassette dialysis (slide-A-Lyzer G2, 2000 MWCO, Thermo-Fisher, Waltham, MA, USA). The product was isolated via freeze-drying. See Supplementary Materials for IR and HNMR spectra.

2.4.2. Using Initiator

A quantity of 150 mg of heparin allyl ester was dissolved in 25 mL water. To the solution was added 15 mg 4,4′-azobis (4-cyanovaleric acid). The stirred solution was heated at 80 °C for 8 h. The solution was added to 100 mL of 5% NaOAc/methanol. The solid was redissolved in 10%NaCl solution and reprecipitated by addition to 3× volume methanol. The solid was dissolved in water and freeze-dried to afford a white solid.

*2.5. Thiol-Ene Reactions of Heparin 4-Vinylbenzyl Ester (**2**) General Procedure*

Heparin 4-vinyl benzyl ester (**2**) and a thiol were dissolved in water or mixture of water and THF (1:1) to which 4,4′-azobis (4-cyanovaleric acid) was added. The solution was irradiated overnight with broadband UV light. While no special efforts were made to control temperature, the temperature of the solution did not rise notably. The resulting solution was transferred into a 3 mL–dialysis cassette (slide-A-Lyzer G2, 2000 MWCO). The dialysis was performed using 1 L water, changing the water once every 12 h for a total of 36 h. The content of the cassette was transferred to a vial and subjected to freeze-drying to obtain a solid product.

2.5.1. Reaction with Cysteine (**4**)

General procedure was followed using heparin 4-vinyl benzyl ester (50 mg) and L-cysteine (178 mg, 1.47 mmol, 20 eq), 1.5 mL of water, and 4,4′-azobis (4-cyanovaleric acid) (18 mg, 0.0018 mmol, 0.25 eq) to furnish white solid (58.1 mg) as the product. See Supplementary Materials for HNMR spectrum.

2.5.2. Reaction with 1-Octanethiol (**5**)

General procedure was followed using heparin 4-vinyl benzyl ester (50 mg, 1 eq) and 1-octanethiol (255 µL, 1.47 mmol, 20 eq), 1.5 mL of water/THF (1:1), and 4,4′-azobis (4-cyanovaleric acid) (18 mg, 0.0018 mmol, 0.25 eq) to furnish white solid (50.4 mg) as the product. See Supplementary Materials for HNMR spectrum.

2.5.3. Reaction with 4-Aminothiophenol (**6**)

General procedure was followed using heparin 4-vinyl benzyl ester (50 mg, 1 eq) and 4-aminothiophenol (184 mg, 1.47 mmol, 20 eq), 1.5 mL of water/THF (1:1), and 4,4′-azobis (4-cyanovaleric acid) (18 mg, 0.0018 mmol, 0.25 eq) to furnish a brown solid (51.3 mg) as the product. See Supplementary Materials for HNMR spectrum.

2.6. Thiol-Ene Reactions of Enoxaparin

2.6.1. Reaction with Mercaptoacetic Acid Using Initiator (**7**)

A quantity of 300 mg enoxaparin was dissolved in 5 mL water. Then, 30 mg 4,4′-azobis (4-cyanovaleric acid) was added followed by 20 mg mercaptoacetic acid. The solution was heated to 80°C with stirring for 18 h then precipitated by addition to 25 mL 5% sodium acetate and methanol. The solid was redissolved in water and freeze dried to yield a transparent film.

2.6.2. Reaction with Mercaptoacetic Acid Using Irradiation

A quantity of 200 mg enoxaparin was placed in a small beaker and dissolved in 5 mL water. Then, 0.12 mL mercaptoacetic acid was added and the stirred solution was irradiated with a UV lamp for 100 min. The solution was placed into a dialysis cassette (MWCO 2000). The dialyzed solution was freeze-dried to a transparent film. See Supplementary Materials for HNMR spectrum as well as spectrum of enoxaparin.

2.6.3. Reaction with Cysteine Using Irradiation (**8**)

A quantity of 175 mg enoxaparin was placed in a small beaker and dissolved in 5 mL water. Then, 150 mg L-cysteine was added and the stirred solution irradiated with a UV lamp for 100 min. The solution was placed into a dialysis cassette (MWCO 2000). The dialyzed solution was freeze-dried to a transparent film. See Supplementary Materials for HNMR spectrum.

3. Results

3.1. Preparation of Heparin Esters (Figure 3)

The alkylation of the benzalkonium salt of heparin proceeded in a homogenous DMF solution. Since the relative size of the benzalkonium counterion is large, the heparin content of the starting material is only about 40%. Thus, material recovery is good for these sodium salts. While the NMR spectrum is discussed below, the IR spectra shows carbonyl stretching frequencies consistent with ester formation (1726 for 1, 1741 for 2).

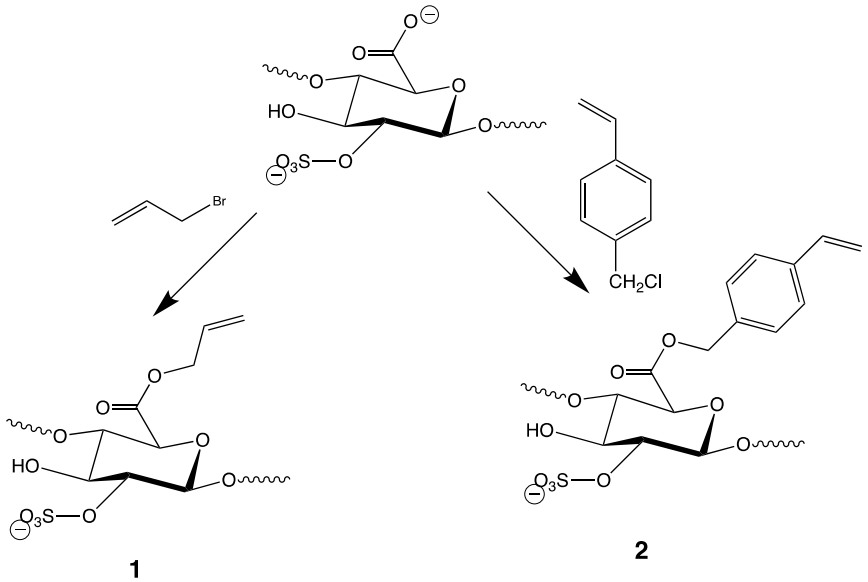

Figure 3. Preparation of heparin esters.

3.2. General Remarks on the Initiation of the Thiol-Ene Reactions

The thiol-ene reaction operates via a free radical process. As such, an initiation step begins the process. The initiation can be carried out by either thermal or photochemical means. For thermal initiation, we chose to use 4,4′-azobis (4-cyanovaleric acid) as the initiator, primarily due to its water solubility. This initiator can also be used as a photolytic initiator. Based on the literature [18] we chose 80°C as a convenient temperature for initiation. Photochemical decomposition takes place at about 405 nm. The use of the above-described broad wavelength source covers that region.

The thiol-ene reaction does not require an independent photoinitiator, but can instead be carried out using direct irradiation of the thiol, normally at about 254 nm (depending on the thiol) [19]. Once again, this wavelength is within the range of the source we used.

*3.3. Thiol-Ene Reaction of Heparin Allyl Ester (**1**) with Mercaptoacetic Acid (Figure 4)*

The reaction proceeded to give the same product under both sets of reaction conditions: irradiation or heating with the water soluble initiator 4,4′-azobis (4-cyanovaleric acid). The retention of the ester linkage was confirmed in the IR spectrum by virtue of a diagnostic signal at 1725 cm^{-1}. NMR analysis confirmed the thiol-ene addition.

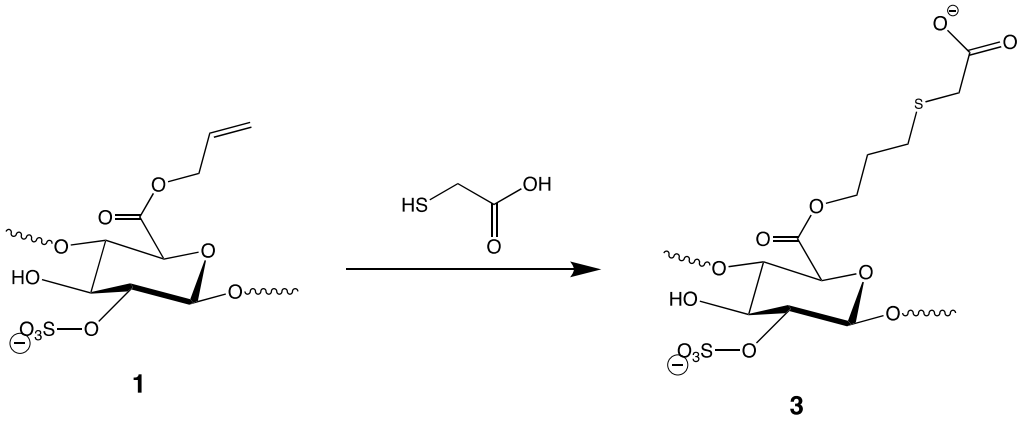

Figure 4. Thiol-ene reaction of [1] with mercaptoacetic acid.

In the allyl ester (**1**), the vinylic signals are located at 6.25, 5.68 and 5.59. The allylic protons on the ester oxygen are mixed with the wide array of anomeric protons in the polymer. COSY analysis of the solution indicates that these protons are diastereotopic with shifts at 5.07 and 4.95. Upon thiol-ene reaction, all of these signals are removed (shifts from the anomeric protons in the 5.0 region remain) (Figure 5). Upfield peaks appear, consistent with the thiol-ene adduct (2.7 for the peaks adjacent to sulfur and 3.7 for adjacent to sulfur and α to the carbonyl, peaks adjacent to oxygen difficult to identify and peaks at 2.0 for the backbone N-acetyl and the central methylene of the linking group).

Upon repeated recording of the NMR spectrum of the thiol-ene product (**3**), the sharper peaks at 1.7 and 2.5 were noted. These peaks increase in intensity as spectra on the same sample are repeated, along with the loss of intensity of the peaks assigned to the thiol-ene adduct. Since the allylic signals do not redevelop, the reaction does not reverse. We conclude that the adduct is labile to hydrolysis in the NMR solution (D_2O). We did not attempt to isolate the hydrolysates, but speculate that heparin would be reformed. Rather than examine conditions that might minimize such hydrolysis, we instead chose to examine an ester that would remove the carboxylate group from proximity to the ester linkage.

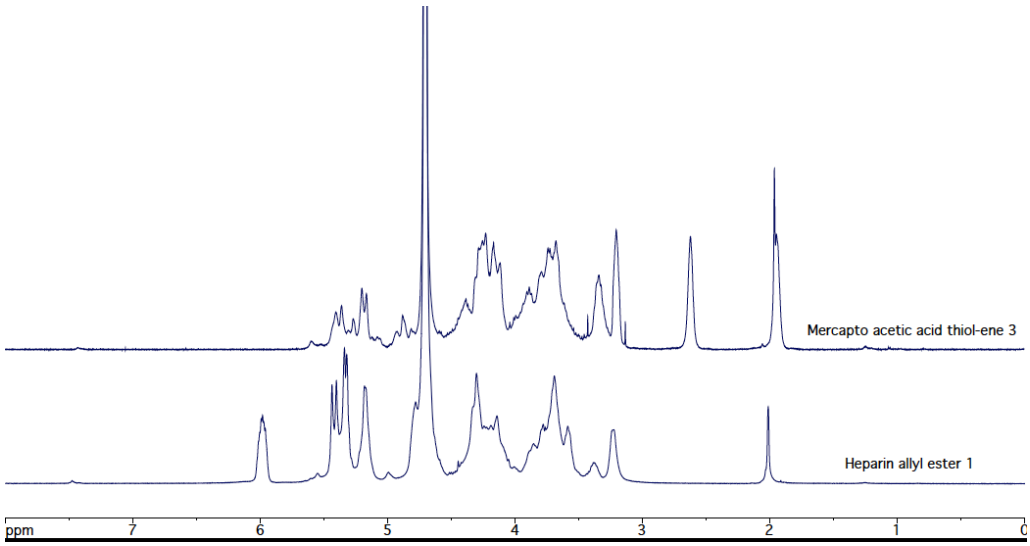

Figure 5. Stacked HNMR spectra of [1] and thiol-ene reaction with mercaptoacetic acid (**3**).

3.4. Thiol-Ene Reactions of Heparin 4-Vinylbenzyl Ester (Figure 6)

Thiol-ene reactions of heparin 4-vinylbenzyl ester were carried out using L-cysteine, octyl mercaptan and 4-aminothiophenol to afford adducts 4, 5, and 6 respectively. NMR analysis of the products establish the conversions (Figure 7).

The starting material (**2**) clearly shows the aromatic ring absorbances centered at 7.5, with the vinylic protons at 6.75 and 5.85. The COSY spectrum indicates the benzylic protons at 5.28. These signals are difficult to discern in the HNMR due to the anomeric proton signals. All of the thiol-ene products retain the aromatic signals, but, as expected, the vinylic protons have been eliminated. In the case of 6, the aromatic region is enhanced, given the introduction of an additional aromatic ring in the adduct. Signals in the upfield region are less easily located, due to the relatively low loading of the ester.

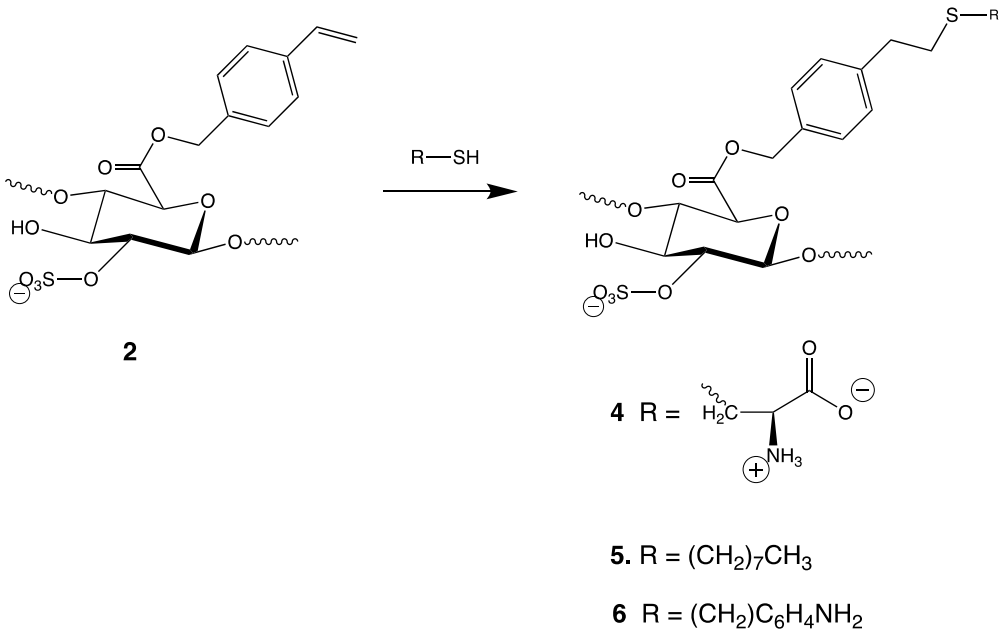

Figure 6. Thiol-ene reactions of heparin 4-vinylbenzyl ester (**2**).

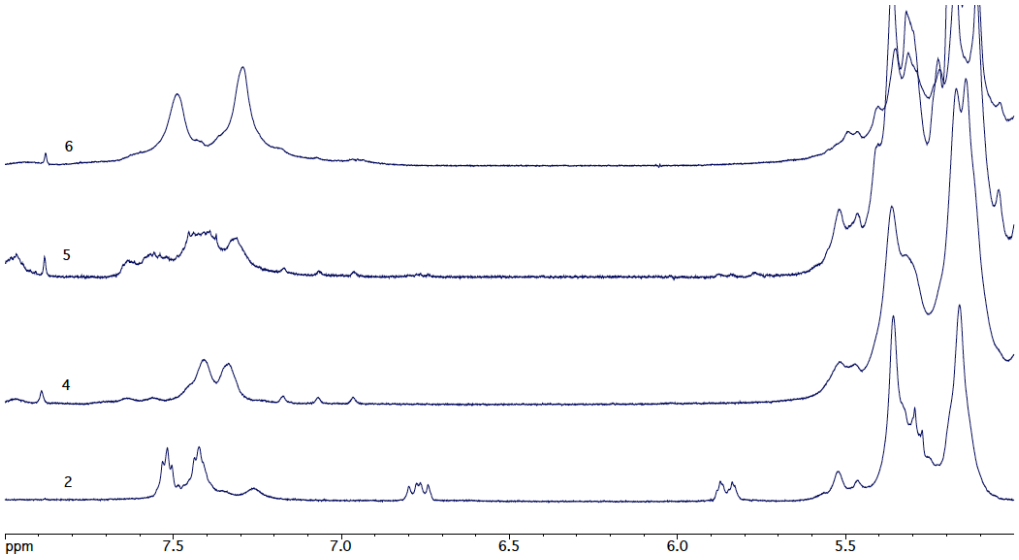

Figure 7. Stacked NMR spectra of 2, 4, 5, 6 in the vinyl and aromatic regions.

3.5. Thiol-Ene Reactions of Enoxaparin (Figure 8)

The NMR signal for the vinyl proton at the non-reducing end of enoxaparin is a narrow doublet at around 5.98. Thiol-ene reaction of mercaptoacetic acid under either initiated or photoactivation results in complete removal of that signal (Figure 9). The same reaction with L-cysteine leads to a reduction, but not a complete removal, of the vinyl signal.

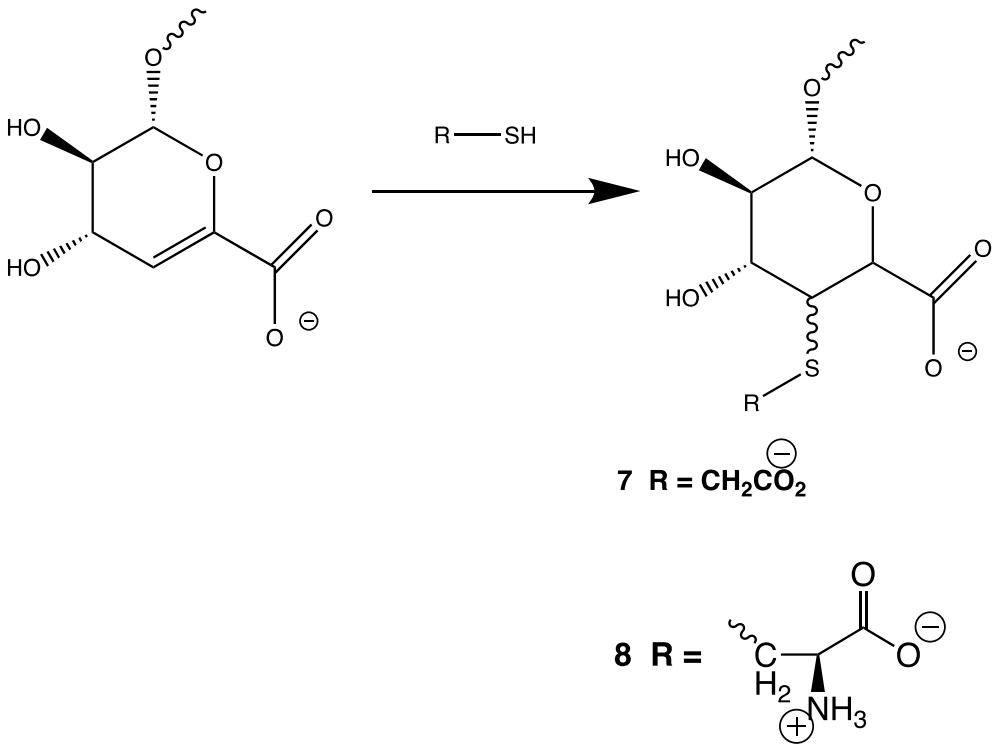

Figure 8. Thiol-ene reactions of enoxaparin.

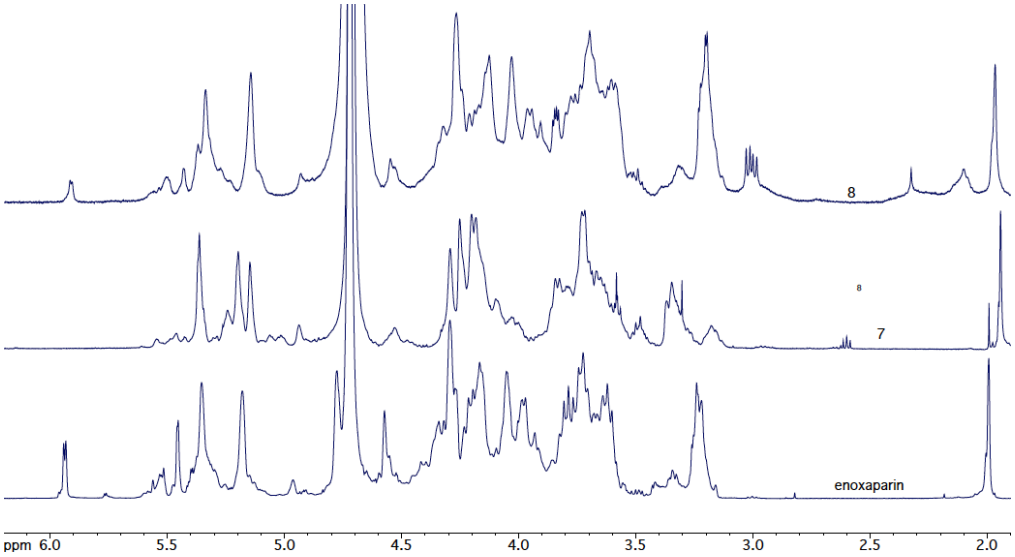

Figure 9. Stacked NMR spectra from 1.9 to 6.2 ppm on enoxaparin, 7 and 8. (Intensities scaled so that NAc peaks near 2.0 are all of equal intensity).

4. Discussion

The thiol-ene reaction is a promising strategy for the conjugation of heparin derivatives. Heparin allyl ester shows a tendency toward hydrolysis, making it less than an ideal candidate, but heparin 4-vinylbenzyl ester shows improved stability in its thiol-ene conjugates. The thiol-ene reaction on the non-reducing end of enoxaparin shows potential for single point conjugation without the need for prior esterification.

From the many mercaptans available for this report, we chose these particular candidates for a variety of reasons. Mercaptoacetic acid and cysteine were chosen as containing functional groups that might reside in biomolecules. As a result, we were able to identify the advantages of the 4-vinylbenzyl ester over the allyl ester. The use of Octyl mercaptan was a successful attempt to conduct the reaction with a less water-soluble mercaptan by using THF as a cosolvent. The use of 4-aminothiophenol extended the use of the reaction to aromatic thiols and to facilitate NMR analysis. Clearly these thiols are descriptive and extensions to other thiols await further research.

5. Patents

The authors have filed a provisional patent application based on this work.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/reactions3030031/s1. Relevant spectra are available as supplemental material, including IR spectrum of heparin allyl ester (**1**); HNMR spectrum of heparin allyl ester (**1**); COSY spectrum of heparin allyl ester (**1**); IR spectrum of heparin 4-vinylbenzyl ester (**2**); HNMR spectrum of heparin 4-vinylbenzyl ester (**2**); COSY spectrum of heparin 4-vinylbenzyl ester (**2**) in vinyl aromatic region; IR spectrum of thiol-ene reaction of heparin allyl ester with mercaptoacetic acid (**3**); HNMR spectrum of thiol-ene reaction of heparin allyl ester with mercaptoacetic acid (**3**); HNMR spectrum of thiol-ene reaction of heparin 4-vinylbenzyl ester with L-cysteine (**4**); HNMR spectrum of thiol-ene reaction of heparin 4-vinylbenzyl ester with octyl mercaptan (**5**); HNMR spectrum of thiol-ene reaction of heparin 4-vinylbenzyl ester with 4-aminothiophenol (**6**); HNMR spectrum of enoxaparin, HNMR spectrum of thiol-ene reaction of enoxaparin and mercaptoacetic acid (**7**); HNMR spectrum of thiol-ene reaction of enoxaparin and L-cysteine (**8**).

Author Contributions: R.T.T. authored the manuscript. Both R.T.T. and D.P.P. developed methodology. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Smithfield Bioscience.

Data Availability Statement: The raw data for the supplemental spectra are available upon request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Stenzel, M.H. Glycopolymers for Drug Delivery: Opportunities and Challenges. *Macromolecules* **2022**, *55*, 4867–4890. [CrossRef]
2. Drira, M.; Hentati, F.; Babich, O.; Sukhikh, S.; Larina, V.; Sharifian, S.; Homai, A.; Fendri, I.; Lemos, M.F.L.; Félix, C.; et al. Bioactive Carbohydrate Polymers—Between Myth and Reality. *Molecules* **2021**, *26*, 7068. [CrossRef] [PubMed]
3. Mycroft-West, C.J.; Su, D.; Pagani, I.; Rudd, T.R.; Elli, S.; Gandhi, N.S.; Guimond, S.E.; Miller, G.J.; Meneghetti, M.C.Z.; Nader, H.B.; et al. Heparin Inhibits Cellular Invasion by SARS-CoV-2: Structural Dependence of the Interaction of the Spike S1 Receptor-Binding Domain with Heparin. *Thromb. Haemost.* **2020**, *120*, 1700–1715. [CrossRef] [PubMed]
4. Poudel, D.P.; Taylor, R.T. Thiol-Ene Click-Inspired Late-Stage Modification of Long-Chain Polyurethane Dendrimers. *Reactions* **2022**, *3*, 12–29. [CrossRef]
5. Hangyeol, C.; Myojeong, K.; Jaebong, J.; Sungwoo, H. Visible-Light-Induced Cysteine-Specific Bioconjugation: Biocompatible Thiol–Ene Click Chemistry. *Angew. Chem. Int. Ed.* **2020**, *59*, 22514–22522. [CrossRef]
6. László, L.; Miklós, N.; Anikó, B.; Pál, H.; Miklós, Z.; Sándor, K. Conjugation of Bioactive Molecules to a Fluorescent Dithiomaleimide by Photoinduced and BEt_3-Initiated Thio-Click Reactions. *Eur. J. Org. Chem.* **2015**, *35*, 7675–7681. [CrossRef]
7. Neumann, K.; Conde-González, A.; Owens, M.; Venturato, A.; Zhang, Y.; Geng, J.; Bradley, M. An Approach to the High-Throughput Fabrication of Glycopolymer Microarrays through Thiol–Ene Chemistry. *Macromolecules* **2017**, *50*, 6026–6031. [CrossRef]
8. Rabenstein, D.L. Heparin and heparan sulfate: Structure and function. *Nat. Prod. Rep.* **2002**, *19*, 312–331. [CrossRef] [PubMed]
9. Danishefsky, I.; Siskovic, R. Heparin derivatives prepared by modification of the uronic acid carboxyl groups. *Thromb. Res.* **1972**, *1*, 173–181. [CrossRef]
10. Ingle, R.G.; Agarwal, A.S. A world of low molecular weight heparins (LMWHs) enoxaparin as a promising moiety—A review. *Carbohydr. Polym.* **2014**, *106*, 148–153. [CrossRef] [PubMed]
11. Fareed, J.; Hoppenstead, D.; Walenga, J.; Iqbal, O.; Ma, Q.; Jeske, W.; Sheikh, T. Pharmacodynamic and Pharmacokinetic Properties of Enoxaparin Implications for Clinical Practice. *Clin. Pharmacokinet.* **2003**, *42*, 1043–1057. [CrossRef] [PubMed]
12. Tandon, R.; Sharp, J.S.; Zhang, F.; Pomin, V.H.; Ashpole, N.M.; Mitra, D.; McCandless, M.G.; Jin, W.; Liu, H.; Sharma, P.; et al. Effective inhibition of SARS-CoV-2 entry by heparin and enoxaparin derivatives. *J. Virol.* **2021**, *95*, e01987-20. [CrossRef] [PubMed]
13. Guerrini, M.; Elli, S.; Gaudesi, D.; Torri, G.; Casu, B.; Mourier, P.; Herman, F.; Boudier, C.; Lorenz, M.; Viskov, C. Effects on Molecular Conformation and Anticoagulant Activities of 1,6-Anhydrosugars at the Reducing Terminal of Antithrombin-Binding Octasaccharides Isolated from Low-Molecular-Weight Heparin Enoxaparin. *J. Med. Chem.* **2010**, *53*, 8030–8040. [CrossRef]
14. Park, J.; Jeon, O.C.; Yun, J.; Nam, H.; Hwang, J.; Al-Hilal, T.A.; Kim, K.; Kim, K.; Byun, Y. End-Site-Specific Conjugation of Enoxaparin and Tetradeoxycholic Acid Using Nonenzymatic Glycosylation for Oral Delivery. *J. Med. Chem.* **2016**, *59*, 10520–10529. [CrossRef]
15. Choubey, A.K.; Dora, C.P.; Bhatt, T.D.; Gill, M.S.; Suresh, S. Development and evaluation of PEGylated Enoxaparin: A novel approach for enhanced anti-Xa activity. *Bioorg. Chem.* **2014**, *54*, 1–6. [CrossRef]
16. Masuko, S.; Higashi, K.; Wang, Z.; Bhaskar, U.; Hickey, A.M.; Zhang, F.; Toida, T.; Dordick, J.S.; Linhardt, R.J. Ozonolysis of the double bond of the unsaturated uronate residue in low-molecular-weight heparin and K5 heparosan. *Carbohydr. Res.* **2011**, *346*, 1962–1966. [CrossRef]
17. Wang, Z.; Shi, C.; Wu, X.; Chen, Y. Efficient access to the non-reducing end of low molecular weight heparin for fluorescent labeling. *Chem. Commun.* **2014**, *50*, 7004–7006. [CrossRef]
18. Zhou, Y.; Zhang, Z.; Postma, A.; Moad, G. Kinetics and mechanism for thermal and photochemical decomposition of 4,4′-azobis(4-cyanopentanoic acid) in aqueous media. *Polym. Chem.* **2019**, *10*, 3284–3287. [CrossRef]
19. Cramer, N.B.; Scott, J.P.; Bowman, C.N. Photopolymerizations of Thiol-Ene Polymers without Photoinitiators. *Macromolecules* **2002**, *35*, 5361–5365. [CrossRef]