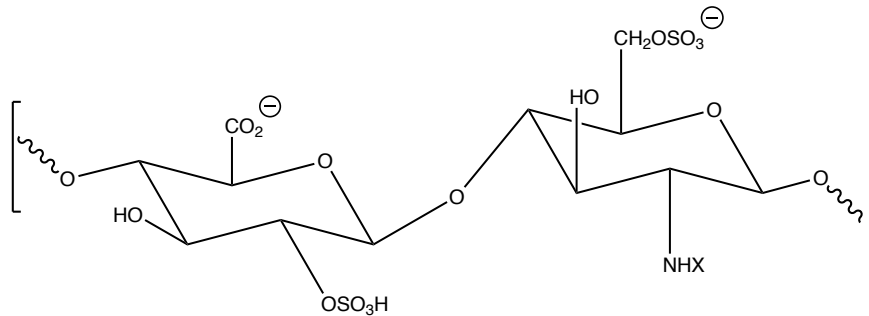# Depolymerization of heparin benzyl ester in a flow system

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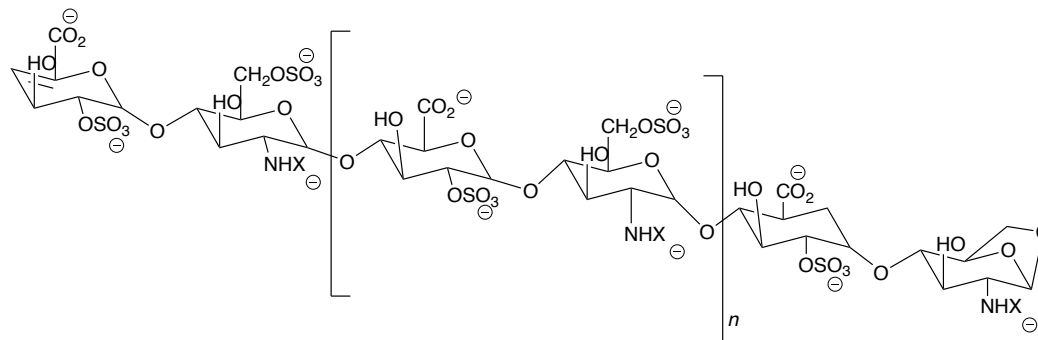Miami University, Oxford, OH 45056 USA

Heparin

- Highly charged glycoaminoglycan, MW ~ 15000
- Binds antithrombin, used as anticoagulant
- Generalized structure below:

Enoxaparin

- Low molecular weight heparin
- Formed by base treatment of heparin benzyl ester
- Can be delivered subcutaneously
- Generalized structure below:

Objective

- Convert current batch processed for depolymerization into continuous flow

- Obtain an enoxaparin-like product

- Develop conditions informed by current batch methods

Protocol

- Syrris FRX flow system with a 12.05 mL reactor coil ($^1/_{16}''$ i.d. tubing)
- T mixer – combine flowing streams of base and heparin benzyl ester solutions
- Neutralize, dilute with methanol, evaporate, dialyze and record HNMR spectrum
- Compare to enoxaparin USP spectrum
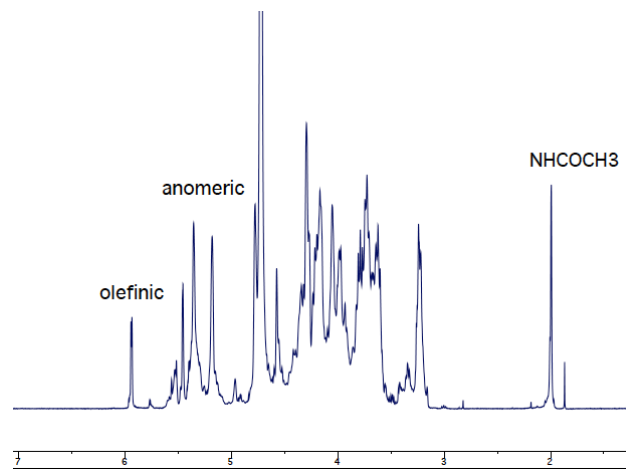
Reaction conditions as varied

Entry	Heparin Ester solution	NaOH solution	Flow ester (mL/min)	Flow base (mL/min]	T (°C)	Reaction Time (min)
1	2g/20mL	2g/20mL	0.25	0.25	70	24.1
2	2g/20mL	2g/20mL	0.12	0.12	50	50.2
3	2g/20mL	2g/20mL	0.12	0.12	65	50.2
4	2g/20mL	2g/20mL	0.12	0.12	75	50.2
5	2g/20mL	2g/20mL	0.12	0.12	65	50.2
6	2g/20mL	2g/20mL	0.24	0.24	65	25.1
7	2g/20mL	2g/20mL	0.12	0.14	55	50.2
8	2g/20mL	2g/20mL	0.24	0.24	75	25.1
9	4g/40mL	4g/40mL	0.24	0.24	65	25.1
10	4g/40mL	4g/40mL	0.24	0.24	55	25.1
11	0.65g/6.5mL	0.65g/6.5mL	0.48	0.48	55	12.6
12	1.0g/20mL	1.0g/20mL	0.24	0.24	55	25.1
13	1.5g/15mL	1.5g/15mL	0.48	0.48	55	12.6

Entry 13 – most enoxaparin-like material
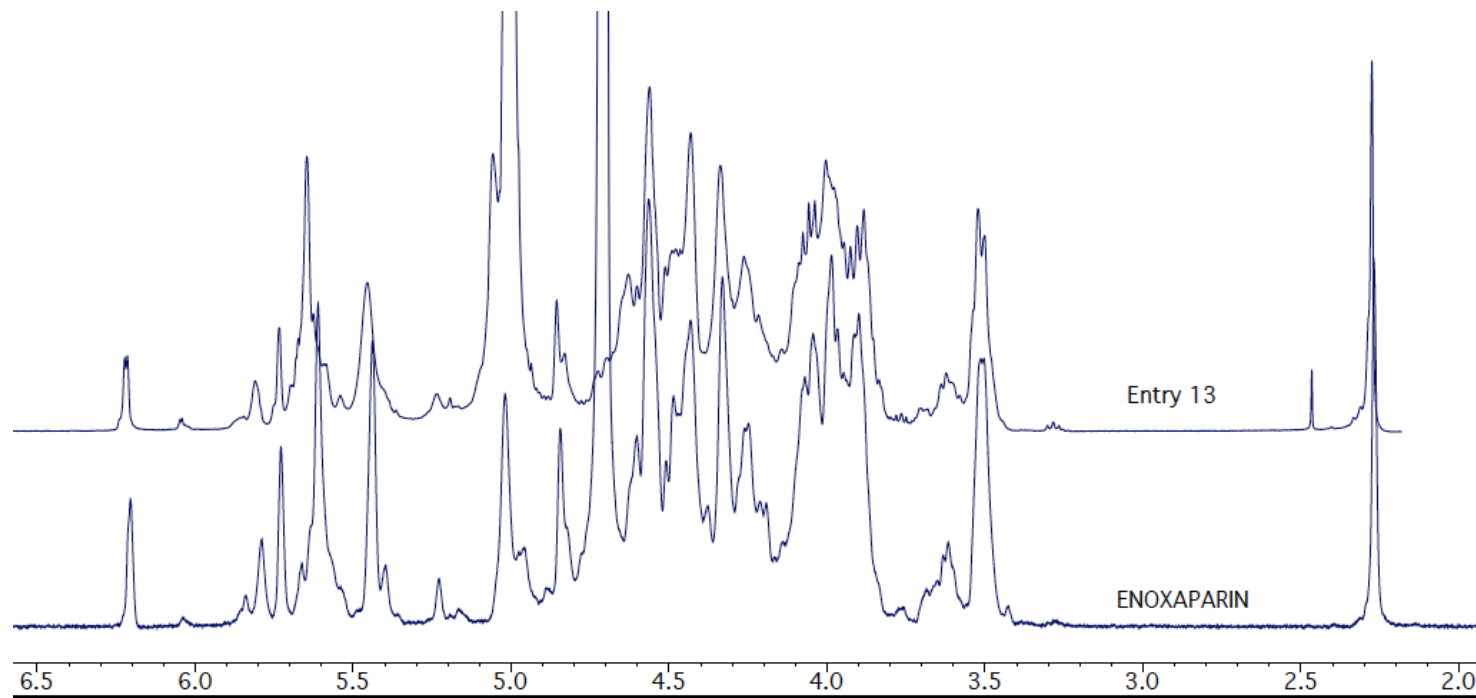
Conclusions

- Base depolymerization of heparin benzyl ester can be converted into a flow process

- Variation of reaction conditions leads to an enoxaparin-like material in the HNMNR spectrum

- Further work to fully convert the purification process into a flow system (desalting, etc.) is needed

Acknowledgement

- Smithfield Bioscience
  - Heparin benzyl ester
  - Support
- Miami University
- Provisional patent on this work has been filed