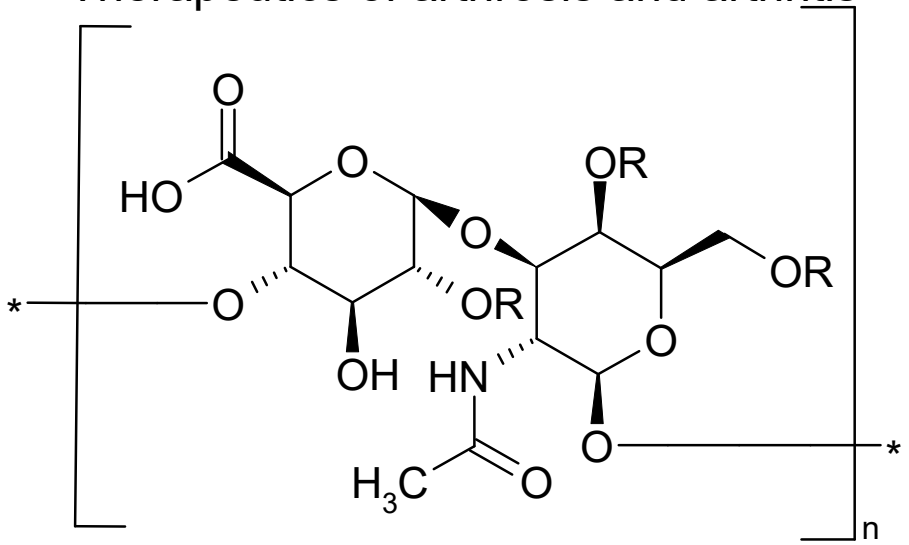
Therapeutic oligo- and polysaccharides

Therapeutics of arthrosis and arthritis



R = -H n or SO_2O^-

chondroitine sulfate

poly-β-D-glucurono-(1→3)-2-deoxy-2-(acetylamino)-β–D-galactopyranose polysulfafe

•prepared by hydrolysis of porcine, bovine or shark cartilages; the composition of the mixture markedly depends on the both raw material and preparation procedure

•absorbed in small intestine in the amount < 10 % probably by an endocytose mechanism

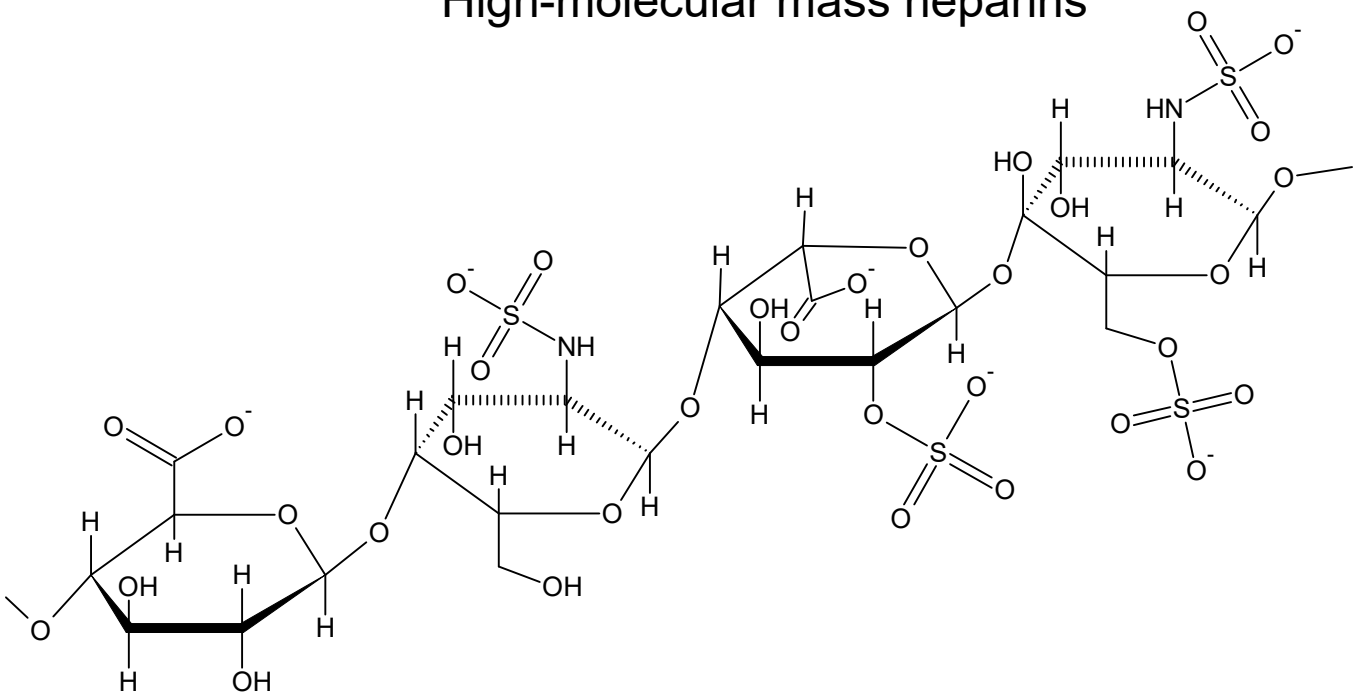
•acts as a prebiotic in the distal part of GIT and is degraded by enzymes of the intestinal microflora into na mono- and disaccharides that are peptidoglycane precursors (namely N-acetylgalactosamine)

•recommended daily dose 800 mg

Authorized drug preparations: Condrosulf ®

Nutraceutics: Mobilin ® , Artryn ® , Gelactiv ® , GS-CS-MSM 3000 ® , GS-Condro (forte) ®, Proenzi 3 ® , Proenzi premium forte 3000 ® -combined with glucosamine and sometimes also with MSM

Anticoagulants
Heparines
High-molecular mass heparins



Heparin chain fragment

β–D-glucuronic acid (α-D-galactosamine) (α-L-iduronic acid) (α-D-glucosamine)

- 6 000 < M_r < 20 000
- < 30 % glucuronic acid
- < 70 % iduronic acid
- *Heparinum natricum, Heparinum calcicum EP*; Heparin Forte Léčiva®
- Mode of action: activates α_2-globuline antitrombin (AT III); heparin-antitrombin complex blocks then blood clotting factors and avoids thrombine formation
- 5 saccharide units suffices for binding to AT III, binding of additional units stabilizes this complex
- isolated from bovine lungs or from intestinal mucous membranes of cattle, pigs or sheep

Anticoagulants
Heparines
Low molecular mass heparines

- EP: a common article *Heparina massae molecularis minoris*
- average M_r < 8000, ≥ 75 % of the total mass must be chains with M_r < 8000
- different selectivity against Xa
- activity must be > 70 IU anti-Xa/mg, selectivity anti-Xa/anti-IIa ≥ 1,5

Low molecular mass heparins (LMWH): production

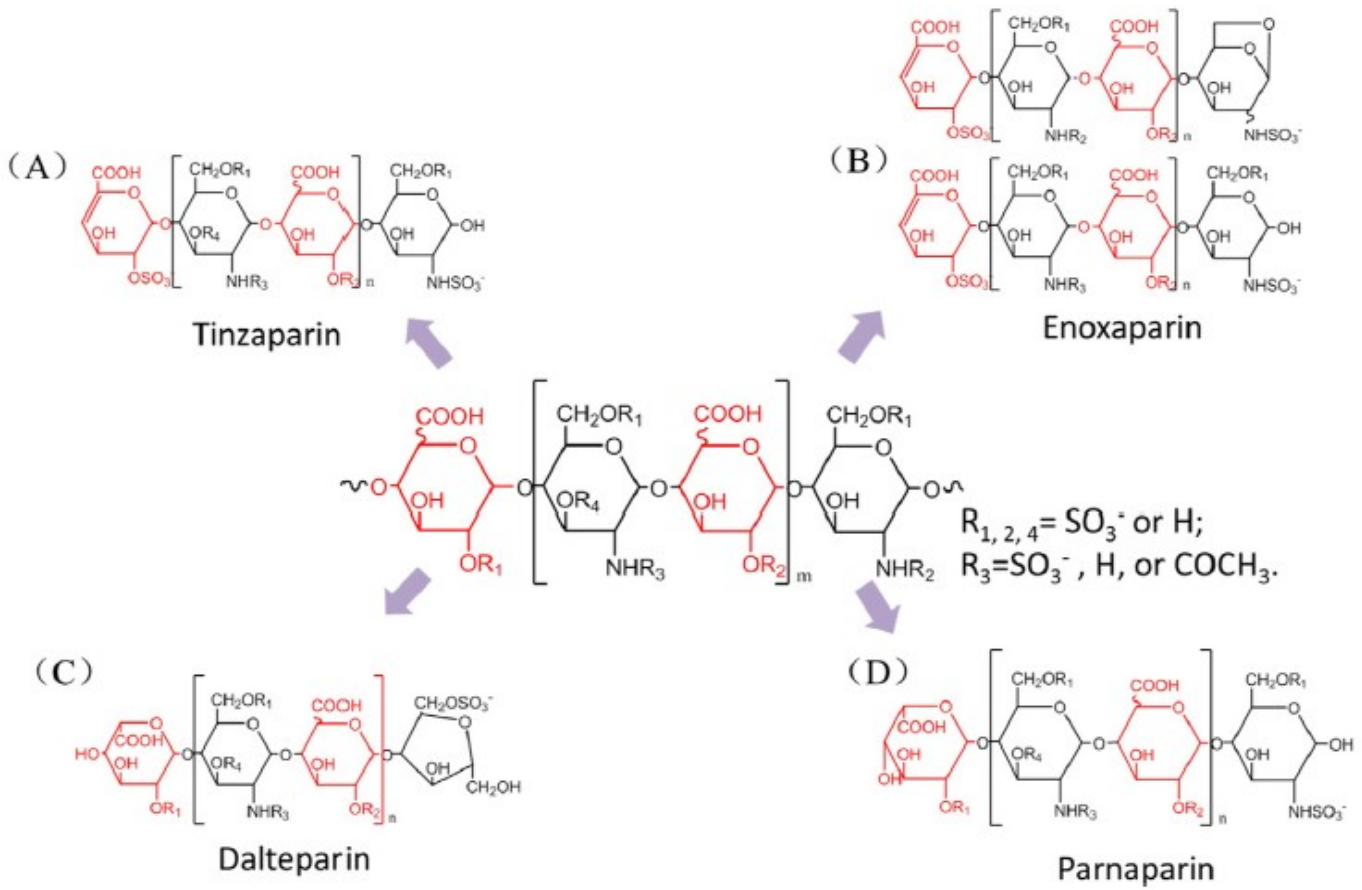
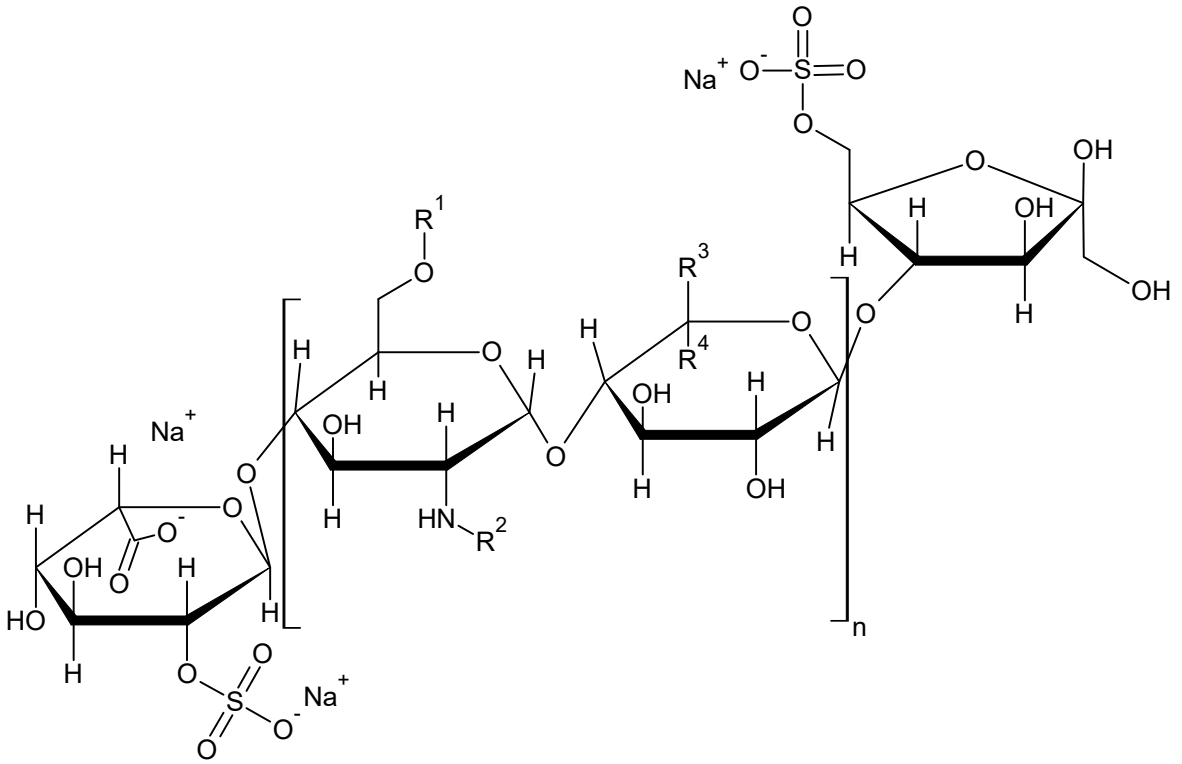


Fig. 2. The representative commercially available LMWHs obtained using different depolymerization methods. Heparin is a long chain constituted of repeating disaccharide building blocks containing 1,4-linked uronic acid (mainly L-iduronic) and D-glucosamine. The depolymerization specificities are dependent on substituents R_1, R_2, R_3 and R_4. (A) Enzymatic depolymerized products by heparinase I, which cleaves sequences containing iduronic acid. (B) LMWHs prepared by alkaline depolymerized reactions. The reaction is a two-step process: firstly, reaction with a benzyl halide to form an intermediate ester and, secondly, hydrolysis following by β-eliminative cleavage under basic conditions. (C) LMWHs obtained by deamination reaction. The specificity of deamination is determined by pH. When pH = 1.5, $R_1 = SO_3^-$ or H, $R_2 = SO_3^-$ or H, $R_3 = SO_3^-$; when pH = 4, $R_1 = SO_3^-$ or H, $R_2 = SO_3^-$ or H, R_3 = H. (D) The majority of H_2O_2 depolymerized heparins have a 2-*O*-sulpho-α-L-idopyranosuronic acid unit at the non-reducing end, and a 2-N,6-*O*-disulpho-D-glucosamine structure at the reducing end of their chains.



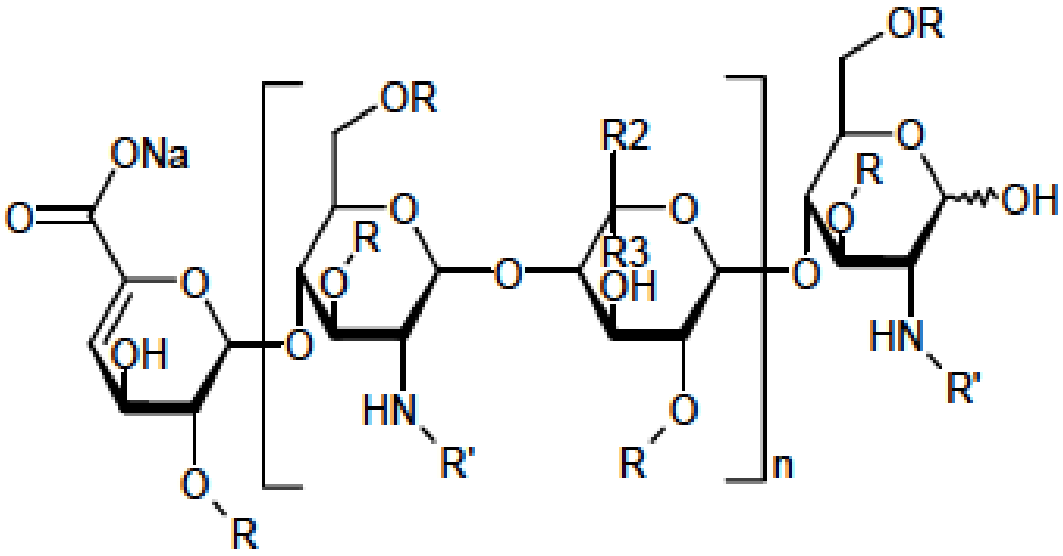
dalteparine sodium salt

Dalteparinum natricum EP

$2 < n < 21$, R^1 = H or SO_3Na, R^2 = SO_3Na or $COCH_3$; R^3 = H and R^4 = COONa or R^3 = COONa and R^4 = H

Fragmin® inj. sol. (*s.c.*)

•treatment of deep venous thromboses and lung embolisms; prevention of blood clotting in an out-of-body system in haemodialysis and haemofiltration linked with an accute renal failure or a chronic renal insufficinecy; prophylaxis of thromboembolism linked with a surgical interventions; treatment of an unstable angina pectoris and non-Q myocardial infarction

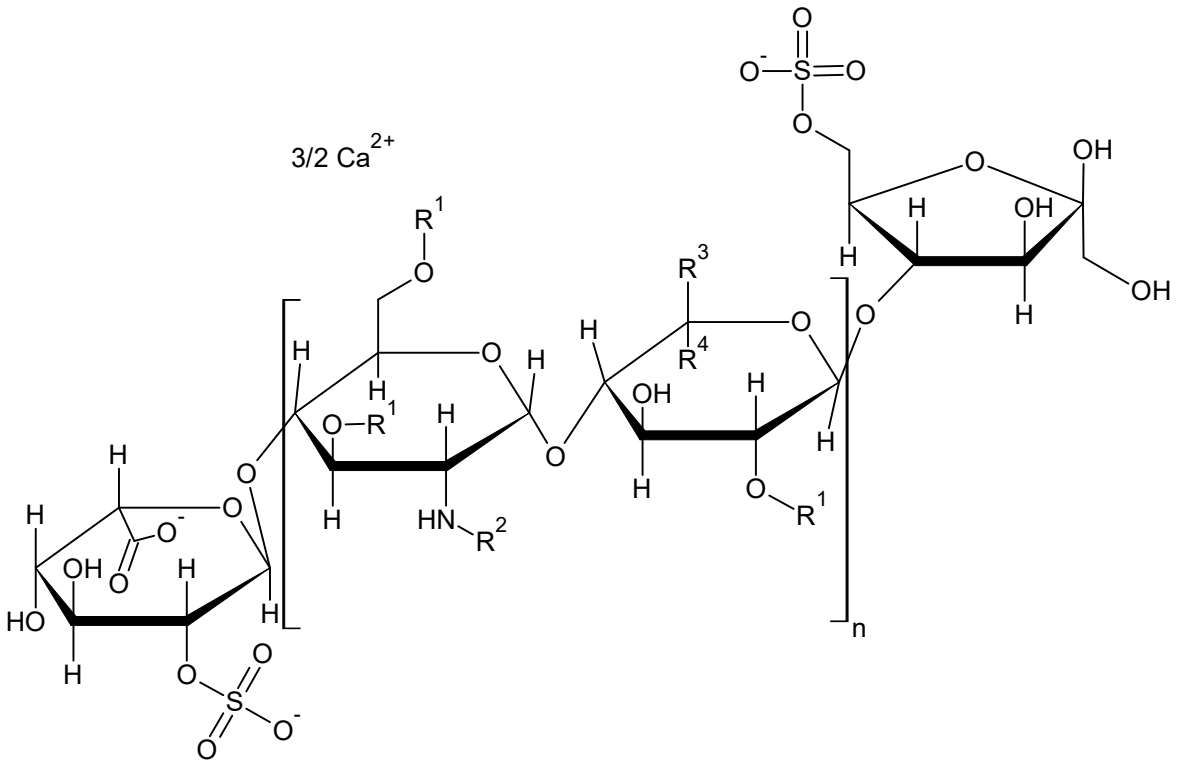


n = 1 to 21, R = H or SO_3Na, R' = H or SO_3Na or $COCH_3$
R2 = H and R3 = CO_2Na or R2 = CO_2Na and R3 = H

enoxaparin sodium

Cruzia ® prefilled syringe (EU), Lovenox ® (USA)

- produced by alkaline depolymerizaton of heparine benzylester
- average M_r cca 4500
- indications: DVT prevention
- in surgical operations
- in internal patients with an acute disease such as heart insuficiency, respiratory failure, severe infections or rheumatic disease with limited movability
- treatment of DVT and pulmonary embolism
- prevention of thrombs formation in and extracorporal circulation during dialysis
- acute coronary syndrom



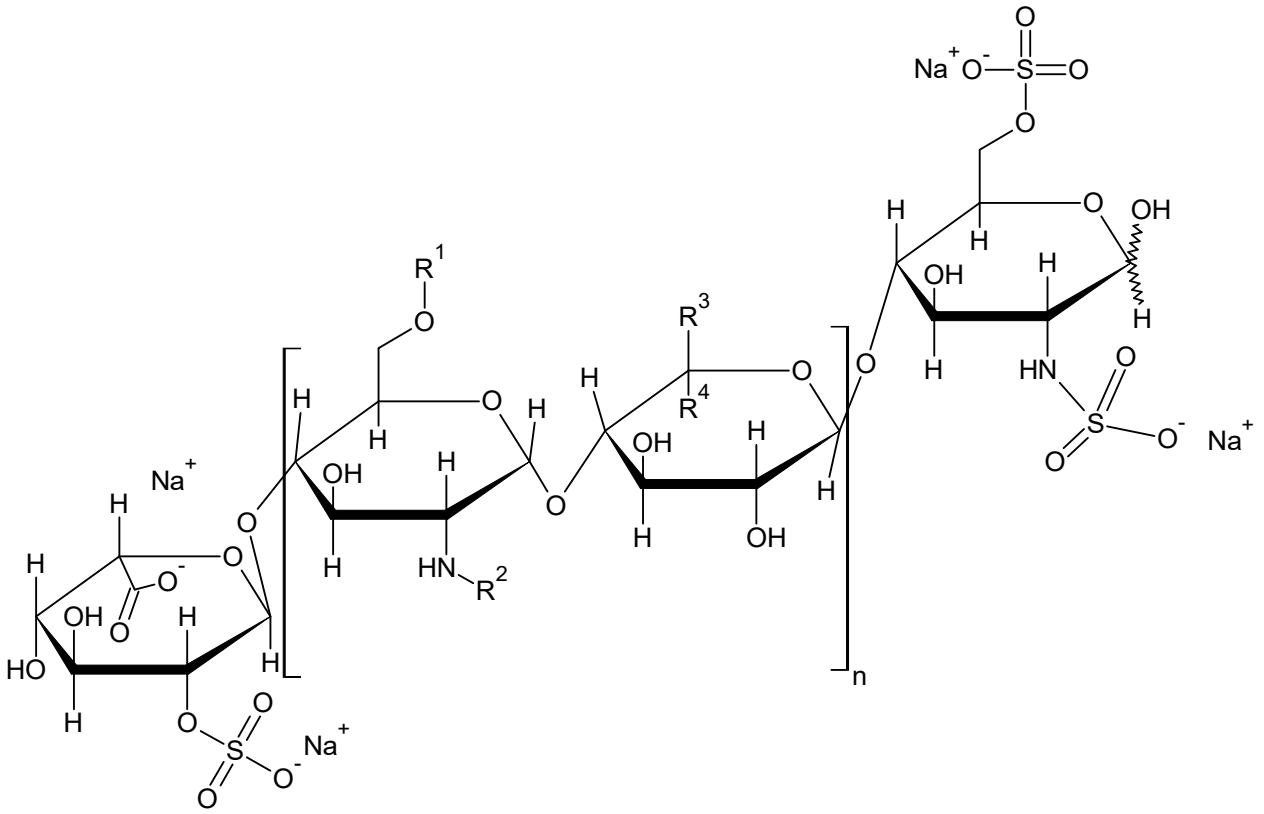
nadroparine calcium salt

Nadroparinum natricum EP

R^1 = H or SO_3(1/2Ca), R^2 = H or SO_3(1/2Ca) or $COCH_3$

R^3 = H and R^4 = COO(1/2Ca) or R^3 = COO(1/2Ca) and R^4 = H

Fraxiparin multi® inj. sol.



parnaparine sodium salt

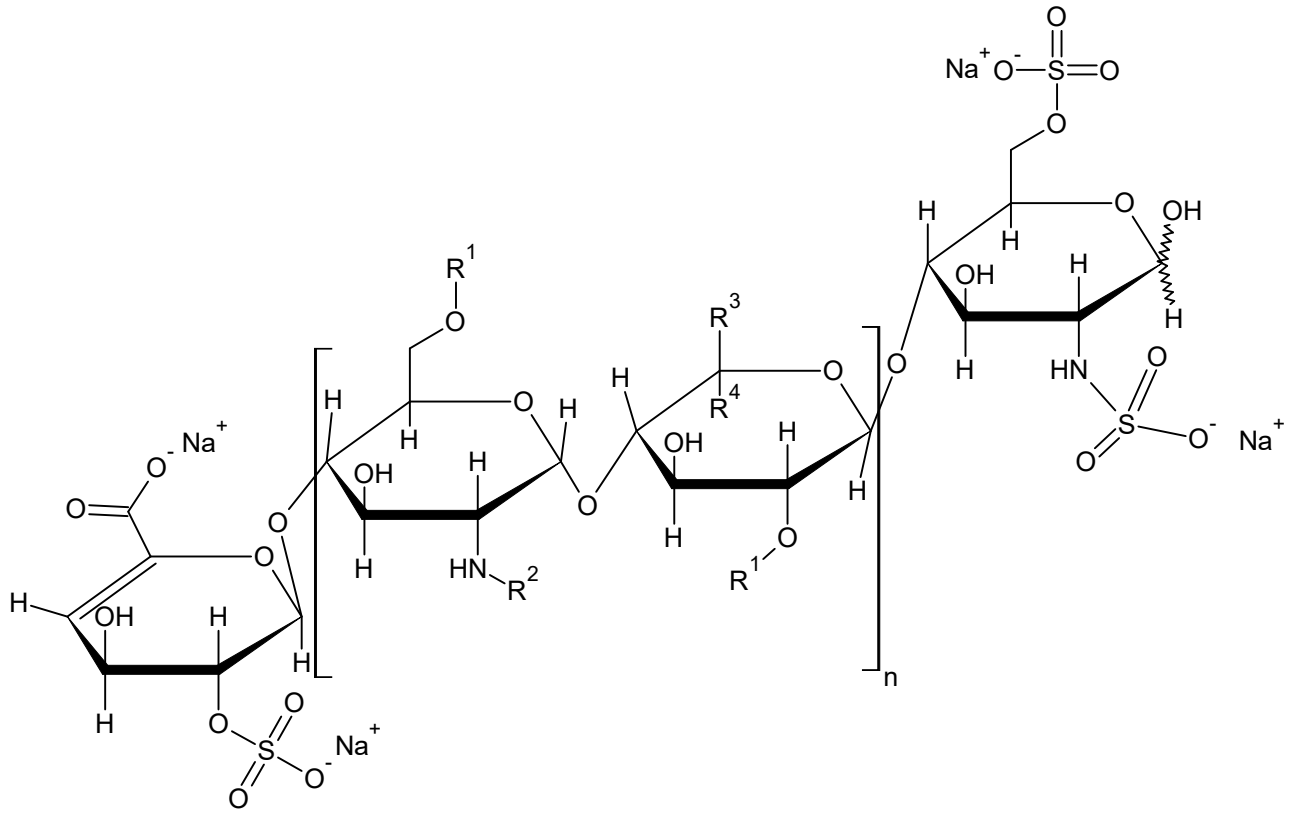
Parnaparinum natricum EP

$0 < n < 22$, R^1 = H or SO_3Na, R^2 = SO_3Na or $COCH_3$ R^3 = H and R^4 = COONa or R^3 = COONa and R^4 = H

•obtained by depolymerization, with hydrogen peroxide and copper (II) acetate or with sodium hypochlorite, of heparins sodium from the healthy edible porcine intestinal mucosa

Fluxum® inj. sol.P

•anti-Xa/anti-IIa between 1.5 and 2.5 (JPXVII)

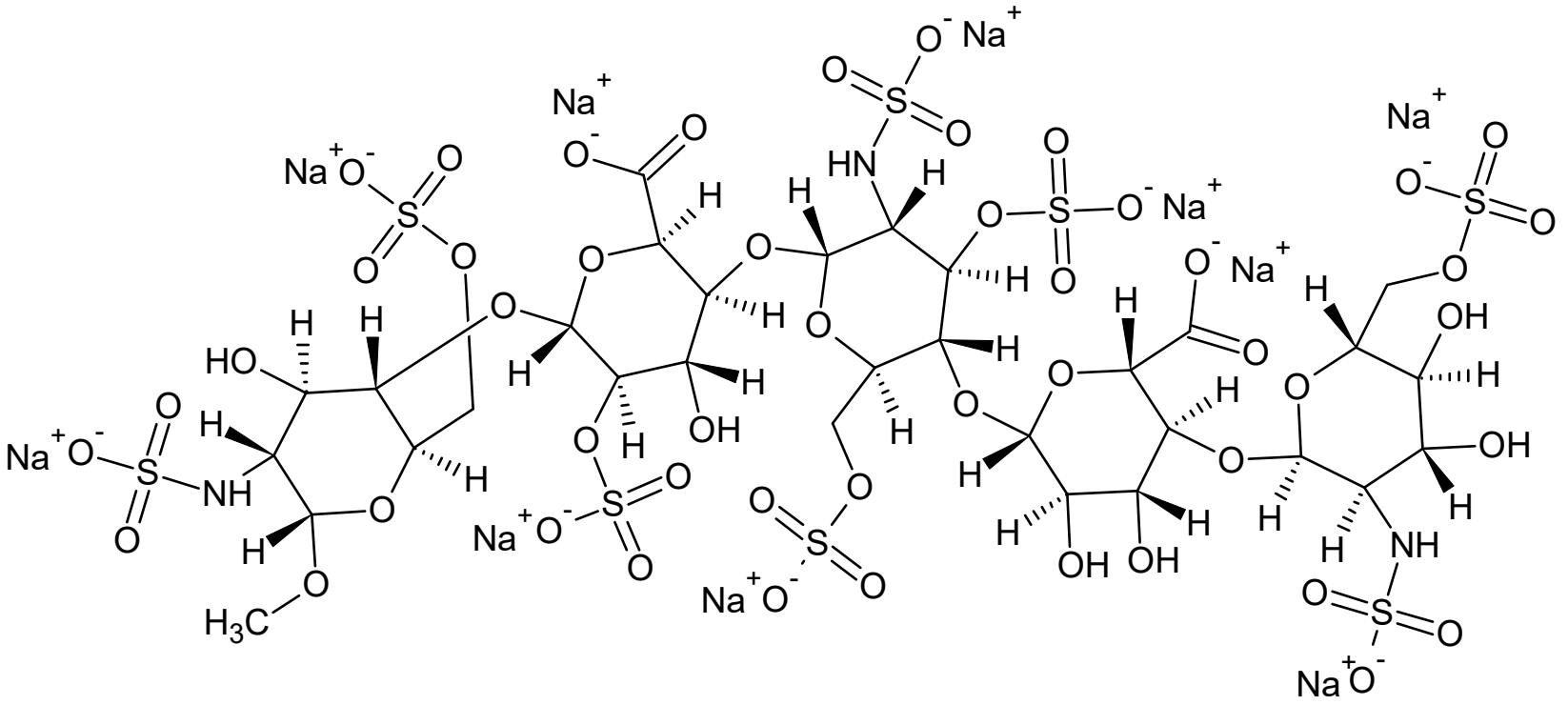


tinzaparine sodium salt

Tinzaparinum natricum EP

$0 < n < 26$, R^1 = H or SO_3Na, R^2 = H or SO_3Na or $COCH_3$, R^3 = H and R^4 = COONa or R^3 = COONa and R^4 = H

Synthetic short chain heparinoids

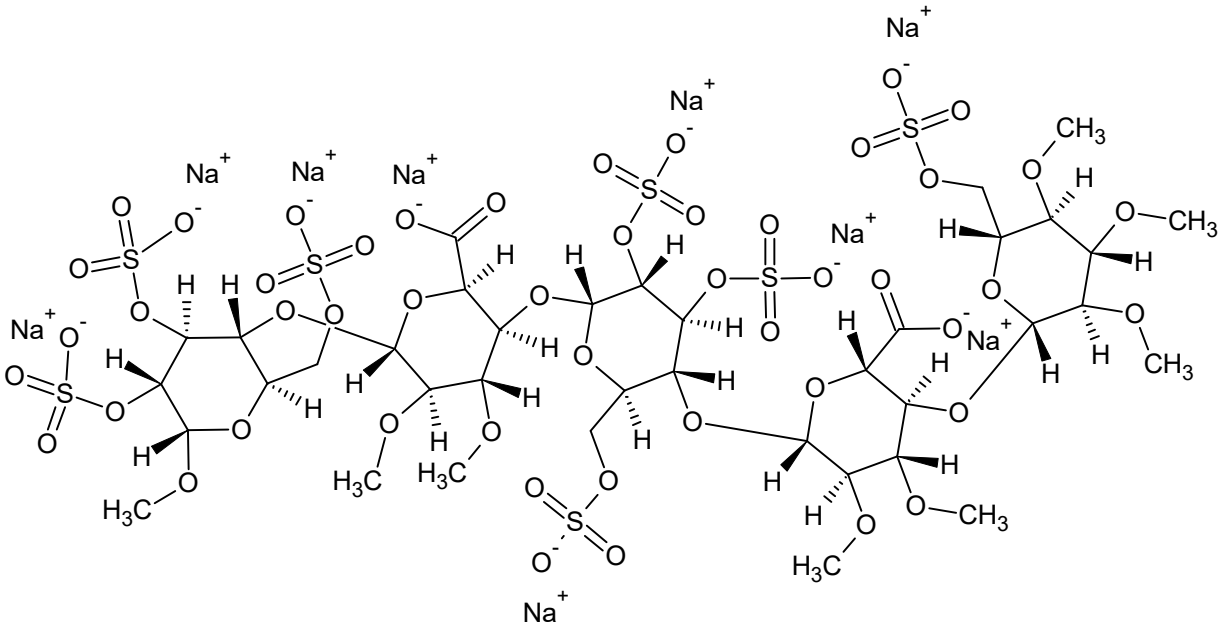


fondaparinux sodium

Arixtra®

- s.c.
- prophylaxis of deep venous thrombosis after fractures, surgical interventions etc.

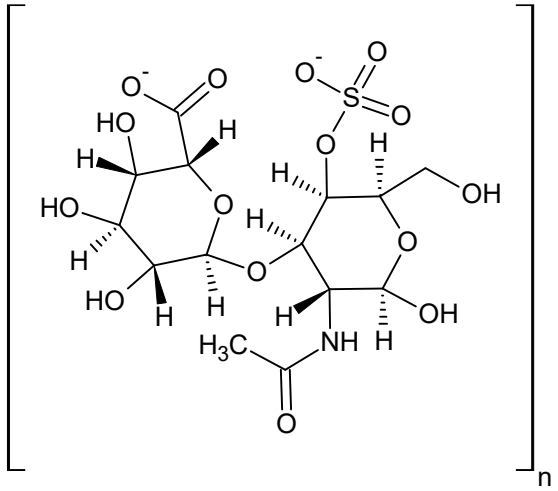
Synthetic short chain heparinoids



idraparinux sodium
syn. SR 34006
•s.c. aplication once weekly only
•phase 3 clinical study for deep venous thrombosis or symptomatic lung embolism

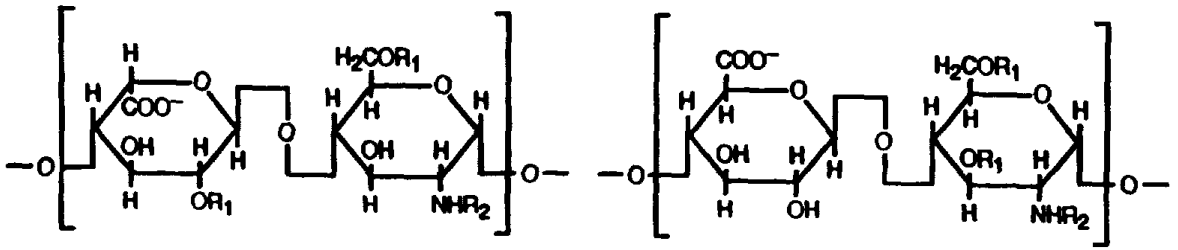
Sulodexide

- a mixture containing 80 % of fast mobility heparine (FMH) and 20 % dermatan sulfate
- FMH fraction is described based on its electrophoretic mobility
- both fractions have lower degree of sulfation and lower anticoagulant activity compared to unfractionized heparin
- obtained from the porcine intestinal mucosa by a patented process
- lower $M_r \Rightarrow$ extensive oral absorption $\Rightarrow$ possibility of **oral administration**
- oral administration results in the release of tissue plasminogen activator and an increase in fibrinolytic activities
- longer $T_{1/2}$
- reduced effect on systemic clotting and bleeding
- treatment of various vascular pathologies including sudden death after MI prevention
- efficiency in treatment of deep vein thrombosis, venous leg ulcers and reduction of reperfusion injury after MI demonstrated by clinical trials
- Vessel Due ® - used
- Sulonex ® - phase 3 and 4 clinical tests for diabetic neuropathy

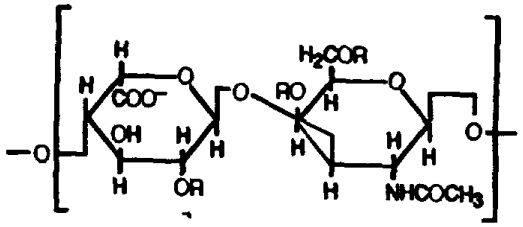


dermatan sulfate

Main Repeating Disaccharide Units:

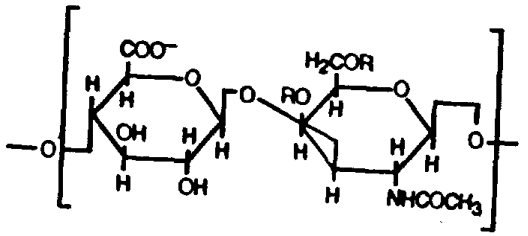


Heparan Sulfate: R_1= H or SO_3^-, R_2= $COCH_3$ or SO_3^-



Dermatan Sulfate

R = H or SO_3^-



Chondroitin Sulfate

danaparoid sodium

Orgaran® *s.c. injections* (reg. USA)

•heparan sulfate produced by *N*-desulfatation followed by *N*-acetylation

•indications: prophylaxis of post operative deep venous thrombosis (DVT), which may lead to pulmonary embolism (PE), in patients undergoing elective hip replacement surgery

•anti-Xa/anti-IIa > 22

Analysis of heparins

^{1}H-NMR

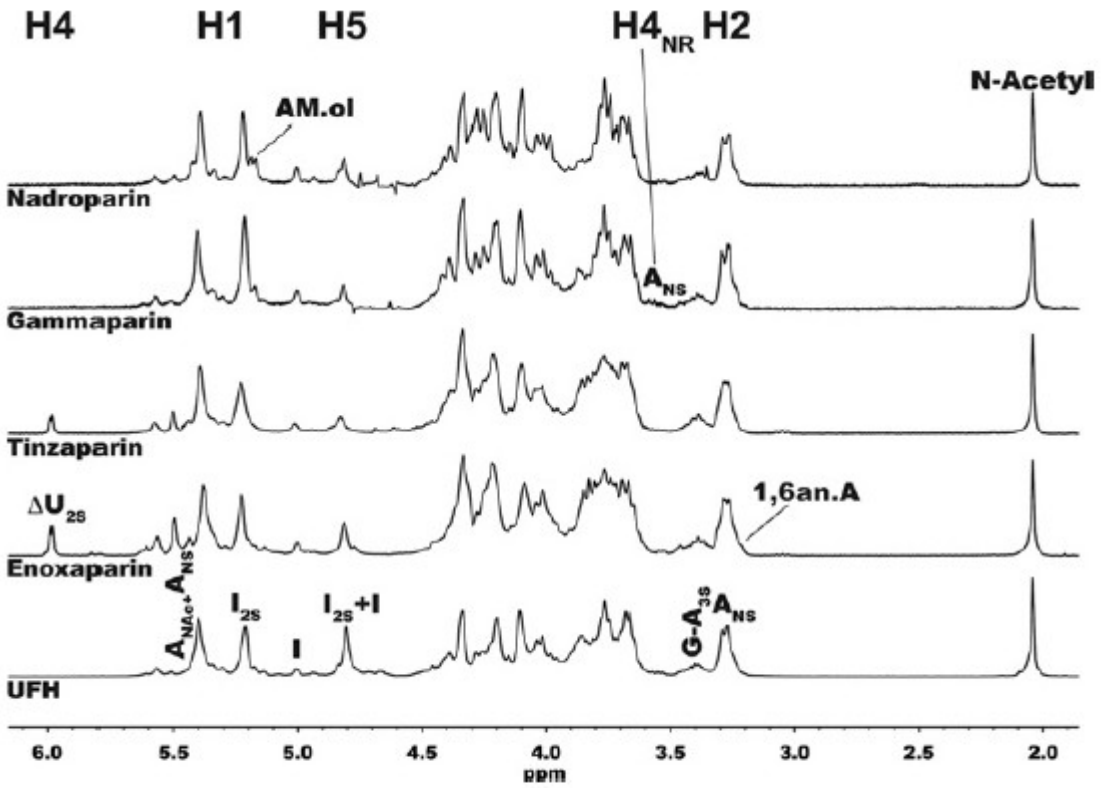


Fig. 3. ^{1}H NMR of UFH and different LMWHs. Major signals are identified. UFH, unfractionated heparin; LMWHs, low molecular weight heparins; A_{NS}, 2-deoxy-2-sulfoamino-D-glucopyranose; I_{2S}, 2-O-sulfo-iduronic acid; G, glucuronic acid; A_{3S}, 2-deoxy-3-O-sulfo-2-amino-D-glucopyranose; A_{NAc}, 2-deoxy-2-acetylamino-D-glucopyranose; 1,6-an.A, 2-amino-1,6-anhydro-2-deoxy-β-D-glucopyranose; AM.ol, 2,5-anhydromannitol; ΔU_{2S}, 2-O-sulfo-4-deoxy-α-L-threo-hex-4-enopyranosil uronic acid, NR, non-reducing end.

- an identification method of EP and JP
  - eg. *Heparinum natricum, Heparinum calcicum*
  - basic frequency ≥ 300 MHz (JP XVII: ≥ 400 MHz)
  - D_2O, referenced to sodium trimethylsilylpropionate
  - according to JP XVII spectral range s DHO signal ± 6 ppm
  - comparison with the spectra of a standard

^{13}C-NMR in accordance with EP 10.0

- 0.2 g of the substance into 0.2 ml D_2O + 0.8 ml H_2O
- 75 MHz
- 40°C
- tube diameter 5 mm
- reference CD_3OD set to 50 ppm

2D-spectra LMWH: HSQC

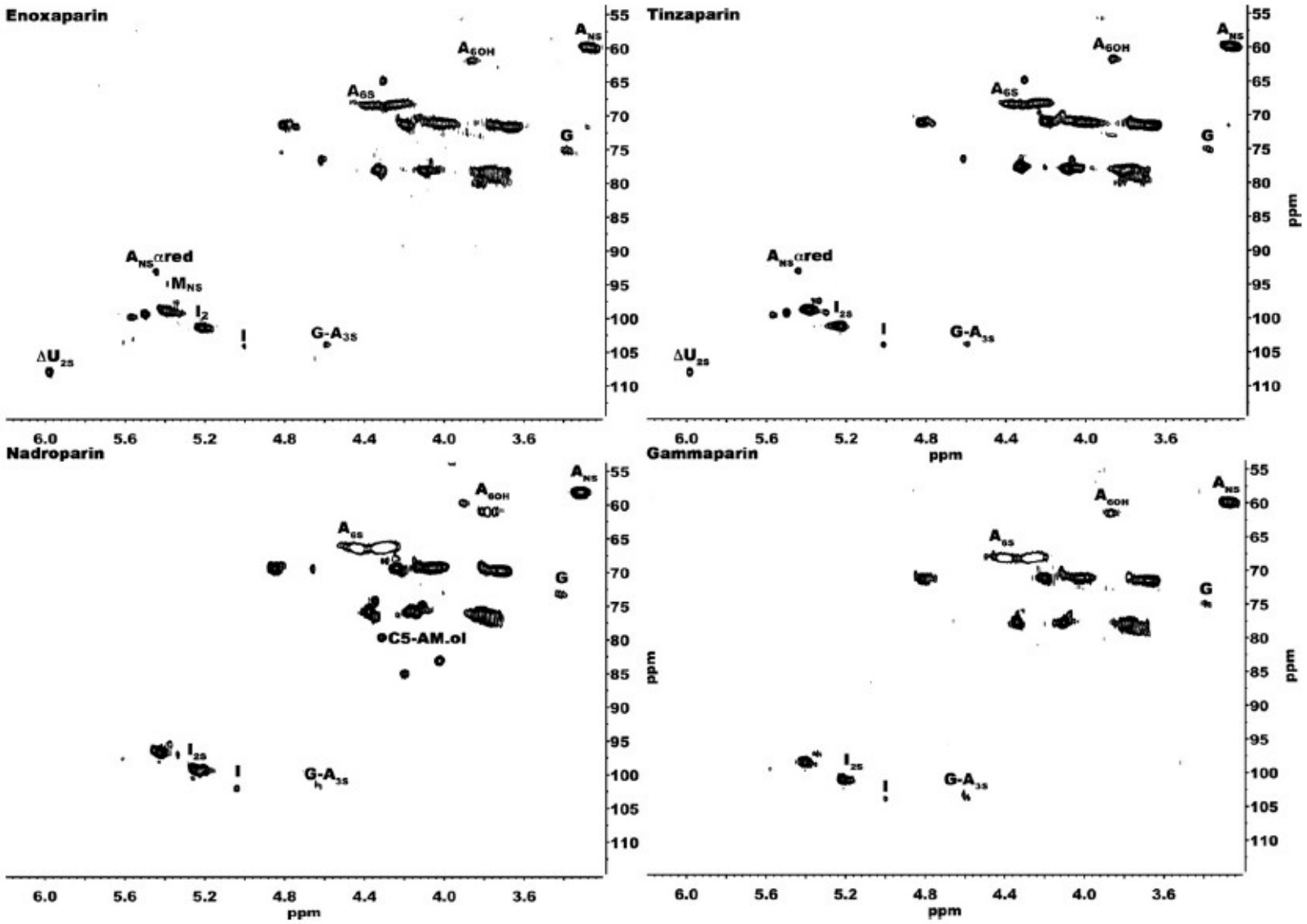


Fig. 4. HSQC spectra of different LMWHs. Signals used for monosaccharide composition are identified. LMWHs, low molecular weight heparins; A_{NS}, 2-deoxy-2-sulfoamino-D-glucopyranose; I_{2S}, 2-O-sulfo-iduronic acid; G, glucuronic acid; A_{3S}, 2-deoxy-3-O-sulfo-2-amino-D-glucopyranose; A_{NAc}, 2-deoxy-2-acetylamino-D-glucopyranose; αred, terminal reducing residue with a configuration; M_{NS}, 2-deoxy-2-sulfamino-D-mannopyranose; 1,6-an.A, 2-amino-1,6-anhydro-2-deoxy-β-D-glucopyranose; 1,6-an.M, 2-amino-1,6-anhydro-2-deoxy-β-D-mannopyranose; AM.ol, 2,5-anhydromannitol; ΔU_{2S}, 2-O-sulfo-4-deoxy-α-L-threo-hex-4-enopyranosil uronic acid; U, Δ4-deoxy-α-L-threo-hex-4-enopyranosil uronic acid.