CLAIMS

1. A method for producing a biosynthetic heparin, comprising:

    a. obtaining a glycosaminoglycan comprising 36-70% of N-sulfated, 2-sulfated, 6-sulfated (NS2S6S) disaccharide group, 6-32% of N-sulfated, 6-sulfated (NS6S) disaccharide group, 0-27% of NS2S group and 1-22% of NS group and having a weight average molecular weight properties appropriate to form a final heparin product of 15,000-19,000 Da, the percentage of heparin chains with a molecular weight of greater than 24,000 Da being not more than 20% of the total, and the ratio of chains between molecular weights of 8,000 to 16,000 Da to chains with molecular weights of 16,000 to 24,000 Da being not less than 1.0;

    b. treating the glycosaminoglycan with an enzyme, which is 3-O-sulfotransferase isoform 1 (3OST-1), in the presence of a sulfate donor;

    to produce a biosynthetic heparin batch.

2. The method of claim 1, wherein the glycosaminoglycan comprises 40-67% of NS2S6S disaccharide group and 6-26% of NS6S disaccharide group

3. The method of claim 1, wherein the glycosaminoglycan comprises 43-64% of NS2S6S disaccharide group and 6-22% of NS6S disaccharide group.

4. The method of claim 1, wherein the glycosaminoglycan comprises 12-27% of NS2S group and 1-17% of NS group.

5. The method of any one of claims 1 to 4, wherein the glycosaminoglycan does not contain 3S disaccharide groups.

6. The method of any one of claims 1 to 5, wherein the glycosaminoglycan further comprises one or more of a OS disaccharide group, a 2S disachharide group, a 6-O-sulfated, NAc (6S) and a 2-O-sulfated, 6-O-sulfated, NAc (2S6S) disaccharide group, and

wherein the combined amount of (a) the OS disaccharide group; (b) the 6S disaccharide group; (c) the 2S disaccharide group ; and (d) the 2S6S dissacharide group is 0.5-28%.

7. The method of any one of claims 1 to 6, wherein in step (b) sufficient glucosamine residues are converted to 3-O-sulfoglucosamine residues so that the produced batch has anti-Xa and anti-IIa activities of no less than 180 units/mg and an anti-Xa/anti-IIa ratio of 0.85-1.15.

8. The method of claim 7, wherein the anti-Xa/anti-IIa ratio is 0.9-1.1.

9. A method of making a third glycosaminoglycan intermediate, which is the glycosaminoglycan as described in any one of claims 1-6:

the method comprising
treating a second glycosaminoglycan intermediate, which is a glycosaminogyclan comprising (a) 44-80% of N-sulfated, 2-sulfated (NS2S) disaccharide group; (b) 13-39% of NS group and (c) 0.4-25% of at least one unmodified N-acetylated glucosamine group selected from OS disaccharide group and 2S disaccharide group, wherein having a weight average molecular weight appropriate to form a final heparin product of 15,000-19,000 Da, the percentage of heparin chains with a molecular weight of greater than 24,000 Da being not more than 20% of the total, and the ratio of chains between molecular weights of 8,000 to 16,000 Da to chains with molecular weights of 16,000 to 24,000 Da being not less than 1.0, with an enzyme, which is 6-O-sulfotransferase isoforms 1 and/or 3 (6OST-1/3), in the presence of a sulfate donor to convert the second glycosaminoglycan intermediate to the third glycosaminoglycan intermediate.

10. The method of claim 9, wherein the second glycosaminoglycan intermediate comprises 44-80% of NS2S group.

11. The method of claim 9, wherein the second glycosaminoglycan intermediate comprises 50-78% of NS2S group.

12. The method of claim 9, wherein the second glycosaminoglycan intermediate comprises 55-77% of NS2S group.

13. The method of claim 9, wherein the second glycosaminoglycan intermediate comprises 60-76% of NS2S group.

14. The method of claim 9, wherein the second glycosaminoglycan intermediate comprises 14-35% of NS group.

15. The method of claim 9, wherein the second glycosaminoglycan intermediate comprises 15-30% of NS group.

16. The method of claim 9, wherein the second glycosaminoglycan intermediate comprises 16-26% of NS group.

17. The method of any one of claims 1-16, wherein the sulfate donor comprises PAPS.

18. The method of claim 17, wherein PAPS is in a solution.

19. The method of any one of claims 1-16, wherein the sulfate donor comprises PAP and PNPS.

20. The method of any one claims 1-16, wherein said treatment is performed in the presence of a recycling system comprising PAPS, PNPS and a catalyst.

21. The method of claim 20, wherein the catalyst is aryl sulfotransferase IV (AST-IV).

22. The method of any one of claims 1-21, wherein the enzyme is in solution.

23. The method of any one of claims 1-21, wherein the enzyme is immobilized.

24. The method of any one of claims 1-16, wherein said treating is performed within an engineered microbial strain containing the genes encoding for NDST, C5-Epi, 2OST, 3OST-1, one or both of 6OST-1 or 6OST-3, and a source of PAPS.