Supplemental material for Analytical Methods

Characterization of currently marketed heparin products: Composition analysis by 2D-NMR.

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Electronic Supplementary Material (ESI) for Analytical Methods

Supplemental Table 1: Calculation of heparin sodium percent monosaccharide from 2D ^{1}H-^{13}C HSQC NMR integrated areas. The chemical shift value shown in parentheses are the signals integrated to obtain peak areas. For illustrative purposes, the mean values obtained from the integration of the spectra from seven lots of heparin sodium are shown.

Signals	**Integrated Crosspeaks ($^{1}H/^{13}C$ chemical shifts)**	**Mean Volume (a.u.)**
A-Ring-C_2	$A_{NS,6x}$(3.27/60.7 ppm)+$A_{NS,3S,6x}$(3.45/59.4 ppm)+$A_{NAc,6x}$(3.92/56.5 ppm)	253 ± 20
A-Ring-C_6	$A_{NS,6S}$(4.27-4.41/69.2 ppm)+$A_{NS,6OH}$(3.85/62.4 ppm)	274 ± 22
A-Ring-C_1	A_{NS}-(G)(5.58/100.5 ppm)+$A_{NS,3S}$(5.52/99.0 ppm)+[$A_{NS,6S}$-(I_{2S})+A_{NAc}-(G)](5.41/99.6 ppm)+$A_{NS,6x}$-(I)(5.34/98.4 ppm)+ A_{NS}-αRed(5.44/94.0 ppm)	285 ± 26
Mean A	Mean of C_2, C_6 and C_1 Areas	272 ± 21
U-Ring-C_1	I_{2S}-($A_{NS,6x}$)(5.22/102.1 ppm)+I-($A_{NS,6S}$)(5.01/105.0 ppm)+I-(A_{NS})(4.95/104.7 ppm)+G-(A_{NS})(4.61/104.8 ppm)+G-(A_{NAc})(4.52/105.3 ppm)+G-($A_{NS,3S}$)(4.61/103.9 ppm)	285 ± 18
%Monosacch.	**Calculation used**	
$A_{NS,6x}$ (C_6)	$A_{NS,6S}$(4.27-4.41/69.2 ppm)÷A-Ring-C_6	
$A_{NS,6OH}$ (C_6)	$A_{NS,6OH}$(3.85/62.4 ppm)÷A-Ring-C_6	
$A_{NS,3S}$ (C_2)	$A_{NS,3S}$(3.45/59.4 ppm)÷A-Ring-C_2	
A_{NAc} (C_2)	A_{NAc}(3.92/56.5 ppm)÷A-Ring-C_2	
A_{NS}-αRed-C_1	A_{NS}-αRed(5.44/94.1 ppm)÷A ring-C_1	
I_{2S}	I_{2S}-($A_{NS,6x}$)(5.22/102.1 ppm)÷U-Ring-C_1	
I_{2OH}	I-($A_{NS,6S}$)(5.01/105.0 ppm)+I-(A_{NS})(4.95/104.7 ppm)÷U-Ring-C_1	
G_{2OH}	G-(A_{NS})(4.61/104.8 ppm)+G-(A_{NAc})(4.52/105.3 ppm)+G-($A_{NS,3S}$)(4.61/103.9 ppm)÷U-Ring-C_1	
Linker (C_1)	Xylose H_1/C_1(4.46/105.8 ppm)÷**{[**U-Ring-C_1+Mean A**]÷2}**	

Supplemental Table 2: Calculation of heparin sodium percent disaccharide composition from only anomeric (H_1/C_1) 2D ^{1}H-^{13}C HSQC-NMR integrated areas. The chemical shift value shown in parentheses are the signals integrated to obtain peak areas.

Signals	**Integrated Crosspeaks ($^{1}H/^{13}C$ chemical shifts)**	**Mean Volume (a.u.)**
A-Ring-C_1	A_{NS}-(G)(5.58/100.5 ppm)+$A_{NS,3S}$(5.52/99.0 ppm)+[$A_{NS,6S}$-(I_{2S})+A_{NAc}-(G)](5.41/99.6 ppm)+$A_{NS,6x}$-(I)(5.34/98.4 ppm)+ A_{NS}-αRed(5.44/94.0 ppm)5.34/98.4 ppm)+ A_{NS}-αRed(5.44/94.1 ppm)	285 ± 26
U-Ring-C_1	I_{2S}-($A_{NS,6x}$)(5.22/102.1 ppm)+I-($A_{NS,6S}$)(5.01/105.0 ppm)+I-(A_{NS})(4.95/104.7 ppm)+G-(A_{NS})(4.61/104.8 ppm)+G-(A_{NAc})(4.52/105.3 ppm)+G-($A_{NS,3S,6x}$)(4.61/103.9 ppm)	285 ± 18
%Disaccharide	**Calculation used**	
$A_{NS,6x}$-(I_{2S})+A_{NAc}-(G)	[$A_{NS,6x}$-(I_{2S})+A_{NAc}-(G)](5.41/99.6 ppm)÷A-Ring-C_1	
$A_{NS,6x}$-(I_{2OH})	$A_{NS,6x}$-(I_{2OH})(5.34/98.4 ppm)÷A-Ring-C_1	
$A_{NS,6x}$-(G)	$A_{NS,6x}$-(G)(5.58/100.5 ppm)÷A-Ring-C_1	
$A_{NS,3S}$-(X)	$A_{NS,3S}$(5.52/99.0 ppm)÷A-Ring-C_1	
I_{2S}-($A_{NS,6x}$)	I_{2S}-($A_{NS,6x}$)(5.22/102.1 ppm)÷U-Ring-C_1	
I_{2OH}-(A_{NS})	I_{2OH}-($A_{NS,6S}$)(5.01/105.0 ppm)÷U-Ring-C_1	
G_{2OH}-(A_{NS})	G_{2OH}-(A_{NS})(4.61/104.8 ppm)÷U-Ring-C_1	
G_{2OH}-($A_{NS,3S}$)	G_{2OH}-($A_{NS,3S}$)(4.61/103.9 ppm)÷U-Ring-C_1	
G_{2OH}-(A_{NAc})	G_{2OH}-(A_{NAc})(4.52/105.3 ppm)÷U-Ring-C_1	

Supplemental Table 3: Calculation of percent monosaccharide in dalteparin from 2D ^{1}H-^{13}C HSQC NMR integrated cross-peak volumes. The chemical shift values in parentheses are the signals integrated. For illustrative purposes, the mean values obtained from the integration of the spectra from three lots of dalteparin are shown.

Signals	**Integrated Crosspeaks ($^{1}H/^{13}C$ chemical shifts)**	**Mean Volume (a.u.)**
A ring-C_2	$A_{NS,6x}$(3.28/60.7 ppm)+$A_{NS,3S,6x}$(3.46/59.4 ppm)+$A_{NAc,6x}$(3.94/56.6 ppm)+AM.ol_{6X}(3.99/85.8 ppm)+Epo-I(3.74/54.2 ppm)	41 ± 2
A ring-C_6	$A_{NS,6S}$(4.27-4.39/69.2 ppm)+A_{NS}(3.87/62.5 ppm)	50 ± 4
A ring-C_1	A_{NS}-(G)(5.58/100.3 ppm)+$A_{NS,3S}$(5.48/99.0 ppm)+[$A_{NS,6S}$-(I_{2S})+A_{NAc}-(G)](5.39/99.4 ppm)+$A_{NS,6x}$-(I)(5.34/98.2 ppm)+AM.ol_{6X}(3.69-3.74/63.6 ppm)	58 ± 2
Avg. A ring	Average of C_6, $C_2$and C_1 Areas	50 ± 2
U/I/G-Ring Area-C_1	I_{2S}-($A_{NS,6x}$)(5.24/102.0 ppm)+I_{2OH}-($A_{NS,6S}$)(5.01/104.8 ppm)+ I_{2OH}-(A_{NS})(4.91/104.5 ppm)+G_{2OH}-($A_{NS,3S}$)(4.61/103.8 ppm)+G_{2OH}-(A_{NS})(4.60/104.6 ppm)+G_{2OH}-(A_{NAc})(4.54/105.0 ppm)	58 ± 2

%Monosacc.	**Calculation Used**
$A_{NS,6S}$-C_6	$A_{NS,6S}$(4.27-4.39/68.8 ppm)÷A ring-C_6
$A_{NS,6OH}$-C_6	$A_{NS,6OH}$(3.87/62.5 ppm)÷A ring-C_6
$A_{NS,3S,6x}$-C_2	$A_{NS,3S,6x}$(3.46/59.4 ppm)÷A ring-C_2
A_{NAc}-C_2	A_{NAc}(3.94/56.6 ppm)÷Avg. A-C_2
AM.ol_{6X}-C_2	AM.ol_{6X}(3.99/85.8 ppm)÷A ring-C_2
AM.ol_{6OH}-C_X	**{[**[AM.ol_{6OH}-C_4(4.24/88.3 ppm)÷(AM.ol_{6OH}(4.24/88.3 ppm)-C_4+AM.ol_{6S}-C_4(4.18/87.4 ppm))]+ [AM.ol_{6OH}-C_5(4.08/84.4 ppm)÷(AM.ol_{6OH}-C_5+AM.ol_{6S}-C_5(4.27/82.2 ppm))**]}÷2•**[AM.ol_{6X}(3.99/85.8 ppm)÷A ring-C_2]
I_{2S}-C_1	I_{2S}(5.24/102.0 ppm)÷U Ring C_1
I_{2OH}-C_1	**[**I_{2OH}-($A_{NS,6S}$)(5.01/104.8 ppm)+I_{2OH}-(A_{NS})(4.91/104.5 ppm)**]**÷U Ring C_1
G_{2OH}-C_1	[G_{2OH}-(A_{NS})(4.60/104.6 ppm)+G_{2OH}-($A_{NS,3S}$)(4.61/103.8 ppm)+G_{2OH}-(A_{NAc})(4.54/105.0 ppm)]÷U Ring C_1
Epo-I-C_2	Epo-I(3.74/54.2 ppm)÷A ring-C_2
Linker-Xyl-C_1	Xyl(4.46/105.7 ppm)÷U-Ring C_1

Supplemental Table 4: Calculation of dalteparin percent disaccharide composition based on 2D ^1H-^{13}C HSQC-NMR H_1/C_1 cross-peak integrated volumes. The chemical shift value shown in parentheses are the signals integrated to obtain volumes.

Signals	**Integrated Crosspeaks ($^1H/^{13}C$ chemical shifts)**	**Mean Volume (a.u.)**
A ring-C_1	A_{NS}-(G)(5.58/100.3 ppm)+$A_{NS,3S,6x}$(5.48/99.0 ppm)+[$A_{NS,6S}$-(I_{2S})+A_{NAc}-(G)](5.39/99.4 ppm)+$A_{NS,6x}$-(I)(5.34/98.2 ppm)+ AM.ol$_{6X}$(3.69-3.74/63.6 ppm)	58 ± 2
U/I/G-Ring Area-C_1	I_{2S}-($A_{NS,6x}$)(5.24/102.0 ppm)+I_{2OH}-($A_{NS,6S}$)(5.01/104.8 ppm)+ I_{2OH}-(A_{NS})(4.91/104.5 ppm)+G_{2OH}-($A_{NS,3S}$)(4.61/103.8 ppm)+G_{2OH}-(A_{NS})(4.60/104.6 ppm)+G_{2OH}-(A_{NAc})(4.54/105.0 ppm)	58 ± 2
Disaccharide	**Calculation used**	
$A_{NS,6x}$-(I_{2S})+A_{NAc}-(G)	[$A_{NS,6x}$-(I_{2S})+A_{NAc}-(G)](5.39/99.4 ppm)÷A-Ring-C_1	
$A_{NS,6x}$-(I_{2OH})	$A_{NS,6x}$-(I_{2OH})(5.34/98.2 ppm)÷A-Ring-C_1	
$A_{NS,6x}$-(G)	$A_{NS,6x}$-(G)(5.58/100.3 ppm)÷A-Ring-C_1	
$A_{NS,3S,6x}$-(x)	$A_{NS,3S,6x}$(5.48/99.0 ppm)÷A-Ring-C_1	
I_{2S}-($A_{NS,6S}$)	I_{2S}-($A_{NS,6x}$)(5.24/102.0 ppm)÷U-Ring-C_1	
I_{2OH}-($A_{NS,6S}$)	I_{2OH}-($A_{NS,6S}$)(5.01/104.8 ppm)÷U-Ring-C_1	
I_{2OH}-($A_{NS,6OH}$)	I_{2OH}-($A_{NS,6OH}$)(4.91/104.5 ppm)÷U-Ring-C_1	
G_{2OH}-(A_{NS})	G_{2OH}-(A_{NS})(4.60/104.6 ppm)÷U-Ring-C_1	
G_{2OH}-($A_{NS,3S}$)	G_{2OH}-($A_{NS,3S}$)(4.61/103.9 ppm)÷U-Ring-C_1	
G_{2OH}-(A_{NAc})	G_{2OH}-(A_{NAc})(4.52/105.3 ppm)÷U-Ring-C_1	

Supplemental Table 5: Calculation of percent monosaccharide in tinzaparin from 2D ^{1}H-^{13}C HSQC NMR cross-peak integrated volumes. The chemical shift values in parentheses are the signals integrated. For illustrative purposes, the mean values obtained from the integration of the spectra from three lots of tinzaparin are shown.

Signals	**Integrated Crosspeaks ($^{1}H/^{13}C$ chemical shifts)**	**Mean Volume (a.u.)**
A ring-C_2	$A_{NS,6x}$(3.28/60.7 ppm)+$A_{NS,3S,6x}$(3.45/59.5 ppm)+$A_{NAc,6x}$(3.93/56.6 ppm)	38 ± 1
A ring-C_6	$A_{NS,6S}$(4.26-4.38/69.1 ppm)+A_{NS}(3.86/62.5 ppm)	59 ± 2
A ring-C_1	A_{NS}-(G)(5.58/100.4 ppm)+$A_{NS,3S,6x}$(5.49/100.4 ppm)+[$A_{NS,6S}$-(I_{2S})+A_{NAc}-(G)](5.39/99.6 ppm)+$A_{NS,6x}$-(I)(5.34/98.2 ppm)+A_{NS}-αRed(5.44/93.9 ppm)+A_{NAc}-αRed(5.20/93.5 ppm)+A_{NS}-βRed(4.71/98.5 ppm)	55 ± 2
Avg. A ring	Average of C_2, C_6 and C_1 Areas	51 ± 1
U/I/G-Ring Area-C_1	ΔU_{2S}(5.50/100.1 ppm)+I_{2S}-($A_{NS,6x}$)(5.23/102.0 ppm)+I_{2OH}-($A_{NS,6S}$)(5.02/104.8 ppm)+I_{2OH}-(A_{NS})(4.95/104.6 ppm)+G_{2OH}-($A_{NS,3S}$)(4.62/103.9 ppm)+G_{2OH}-(A_{NS})(4.60/104.7 ppm)+G_{2OH}-(A_{NAc})(4.51/105.1 ppm)	58 ± 1
%Monosacc.	**Calculation Used**	
$A_{NS,6S}$-C_6	$A_{NS,6S}$(4.26-4.38/69.1 ppm)÷A ring-C_6	
$A_{NS,6OH}$-C_6	$A_{NS,6OH}$(3.85/62.4 ppm)÷A ring-C_6	
$A_{NS,3S}$-C_2	$A_{NS,3S,6X}$(3.45/59.4 ppm)÷A ring-C_2	
A_{NAc}-C_2	A_{NAc}(3.93/56.6 ppm)÷ A ring-C_2	
A_{NS}-αRed-C_1	A_{NS}-αRed(5.44/93.9 ppm)÷A ring-C_1	
A_{NAc}-αRed-C_1	A_{NAc}-αRed(5.20/93.5 ppm)÷A ring-C_1	
A_{NS}-βRed-C_1	A_{NS}-βRed(4.71/98.5 ppm)÷A ring-C_1	
I_{2S}-C_1	I_{2S}(5.23/102.0 ppm)÷U Ring C_1	
I_{2OH}-C_1	[I_{2OH}-($A_{NS,6S}$)(5.02/104.8 ppm)+I_{2OH}-(A_{NS})(4.95/104.6 ppm)]	
G_{2OH}-C_1	G_{2OH}-($A_{NS,3S}$)(4.62/103.9 ppm)+G_{2OH}-(A_{NS})(4.60/104.7 ppm)+G_{2OH}-(A_{NAc})(4.51/105.1 ppm)	
ΔU_{2S}-C1	ΔU_{2S} C_1(5.50/100.1 ppm)÷U-Ring C_1	
Linker-Xyl-C_1	Xyl (4.48/105.4 ppm)÷U-Ring C_1	

Supplemental Table 6: Calculation of tinzaparin disaccharide composition based on 2D ^{1}H-^{13}C HSQC NMR H_1/C_1 cross-peak integrated volumes. The chemical shift value shown in parentheses are the signals integrated to obtain volumes.

Signals	Integrated Crosspeaks ($^{1}H/^{13}C$ chemical shifts)	Mean Volume (a.u.)
A-Ring-C_1 (n=3)	A_{NS}-(G)(5.58/100.4 ppm)+$A_{NS,3S,6x}$(5.49/99.1 ppm)+[$A_{NS,6S}$-(I_{2S})+A_{NAc}-(G)](5.39/99.6 ppm)+$A_{NS,6x}$-(I)(5.34/98.2 ppm)+A_{NS}-αRed(5.44/93.9 ppm)+A_{NAc}-αRed(5.20/93.5 ppm)+A_{NS}-βRed(4.71/98.5 ppm)	55 ± 2
U/I/G-Ring-C_1 (n=3)	ΔU_{2S}(5.50/100.1 ppm)+ I_{2S}-($A_{NS,6x}$)(5.23/102.0 ppm)+I_{2OH}-($A_{NS,6S}$)(5.02/104.8 ppm)+I_{2OH}-(A_{NS})(4.95/104.6 ppm)+G_{2OH}-($A_{NS,3S}$)(4.62/103.9 ppm)+G_{2OH}-(A_{NS})(4.60/104.7 ppm)+G_{2OH}-(A_{NAc})(4.51/105.1 ppm)	58 ± 1
%Disaccharide	**Calculation used**	
$A_{NS,6x}$-(I_{2S})+A_{NAc}-(G)	[$A_{NS,6x}$-(I_{2S})+A_{NAc}-(G)](5.39/99.6 ppm)÷A-Ring-C_1	
$A_{NS,6x}$-(I_{2OH})	$A_{NS,6x}$-(I_{2OH})(5.34/98.2 ppm)÷A-Ring-C_1	
$A_{NS,6x}$-(G)	$A_{NS,6x}$-(G)(5.58/100.4 ppm)÷A-Ring-C_1	
$A_{NS,3S,6x}$-(x)	$A_{NS,3S,6x}$(5.49/99.1 ppm)÷A-Ring-C_1	
I_{2S}-($A_{NS,6S}$)	I_{2S}-($A_{NS,6x}$)(5.23/102.0 ppm)÷U-Ring-C_1	
I_{2OH}-($A_{NS,6S}$)	I_{2OH}-($A_{NS,6S}$)(5.02/104.8 ppm)÷U-Ring-C_1	
I_{2OH}-($A_{NS,6OH}$)	I_{2OH}-($A_{NS,6OH}$)(4.95/104.6 ppm)÷U-Ring-C_1	
G_{2OH}-(A_{NS})	G_{2OH}-(A_{NS})(4.60/104.7 ppm)÷U-Ring-C_1	
G_{2OH}-($A_{NS,3S}$)	G_{2OH}-($A_{NS,3S}$)(4.62/103.9 ppm)÷U-Ring-C_1	
G_{2OH}-(A_{NAc})	G_{2OH}-(A_{NAc})(4.51/105.1 ppm)÷U-Ring-C_1	
ΔU_{2S}-(A)	ΔU_{2S}(5.50/100.1 ppm)÷U-Ring-C_1	

Supplemental Table 7: Calculation of percent monosaccharide composition of enoxaparin from 2D ^{1}H-^{13}C HSQC NMR cross peak integrated volumes. The chemical shift values in parentheses are the signals integrated. For illustrative purposes, the mean values obtained from the integration of the spectra from four lots of enoxaparin are shown.

Signals	**Integrated Crosspeaks ($^{1}H/^{13}C$ chemical shifts)**	**Mean**
A ring-C_2	$A_{NS,6x}$(3.28/60.7 ppm)+$A_{NS,3S,6x}$(3.46/59.4 ppm)+$A_{NAc,6x}$(3.92/56.5 ppm)+1,6-an.A(3.21/58.3 ppm)+1,6-an.M(3.46/55.0 ppm)+M_{NS}-αRed(3.61/60.2 ppm)+Epo-I(3.73/54.2 ppm)	40 ± 3
A ring-C_6	$A_{NS,6S}$(4.28-4.38/69.1 ppm)+A_{NS}(3.85/62.5 ppm)+1,6-an.A(3.78/67.4 ppm)+1,6-an.M(3.80/68.0 ppm)	57 ± 5
A ring-C_1	A_{NS}-(G)(5.56/100.4 ppm)+$A_{NS,3S,6x}$(5.48/99.0 ppm)+[$A_{NS,6S}$-(I_{2S})+A_{NAc}-(G)](5.37/99.6 ppm)+$A_{NS,6x}$-(I)(5.34/98.2 ppm)+1,6-an.A(5.61/104.1 ppm)+1,6-an.M(5.56/103.7 ppm)+M_{NS}-αRed(5.39/95.6 ppm)+A_{NS}-αRed(5.44/93.9 ppm)+A_{NAc}-αRed(5.20/93.4 ppm)+A_{NS}-βRed(4.71/98.5 ppm)	59 ± 5
Avg. A ring	Average of C_2, C_6 and C_1 Areas	52 ± 4
U/I/G-ring Area-C_1	ΔU_{2S}(5.50/100.1 ppm)+ΔU_{2OH}(5.16/103.7 ppm)+I_{2S}-($A_{NS,6x}$)(5.22/102.0 ppm)+I_{2OH}-($A_{NS,6S}$)(5.00/104.8 ppm)+I_{2OH}-(A_{NS})(4.94/104.5 ppm)+G_{2S}-(A_{NS})(4.73/102.9 ppm)+G_{2OH}-($A_{NS,3S}$)(4.61/103.8 ppm)+G_{2OH}-(A_{NS})(4.60/104.6 ppm)+G_{2OH}-(A_{NAc})(4.51/105.0 ppm)+I_{2S}-βRed (4.99/94.6 ppm)+I_{2S}-αRed (5.42/95.4 ppm)	61 ± 5

%Monosac.	**Calculation Used**
$A_{NS,6S}$-C_6	$A_{NS,6S}$(4.28-4.38/69.1 ppm)÷A ring-C_6
$A_{NS,6OH}$-C_6	$A_{NS,6OH}$(3.85/62.4 ppm)÷A ring-C_6
$A_{NS,3S}$-C_2	$A_{NS,3S,6X}$(3.46/59.4 ppm)÷A ring-C_2
A_{NAc}-C_2	$A_{NAc,6x}$(3.92/56.5 ppm)÷A ring-C_2
A_{NS}-αRed-C_1	A_{NS}-αRed(5.44/93.9 ppm)÷A ring-C_1
A_{NAc}-αRed-C_1	A_{NAc}-αRed(5.20/93.4 ppm)÷A ring-C_1
A_{NS}-βRed-C_1	A_{NS}-βRed(4.71/98.5 ppm)÷A ring-C_1
M_{NS}-αRed-C_1	M_{NS}-αRed(5.39/95.6 ppm)÷A ring-C_1
1,6-an.A-C_1	1,6-an.A(5.61/104.1 ppm)÷A ring-C_1
1,6-an.M-C_1	1,6-an.M(5.56/103.7 ppm)÷A ring-C_1
I_{2S}-C_1	I_{2S}(5.22/102.0 ppm)÷U Ring C_1
I_{2OH}-C_1	**[**I_{2OH}-($A_{NS,6S}$)(5.00/104.8 ppm)+I_{2OH}-(A_{NS})(4.94/104.5 ppm)**]**÷U-Ring C_1
G_{2S}-C_1	G_{2S}-(A_{NS})(4.73/102.9 ppm)÷U-Ring C_1
G_{2OH}-C_1	G_{2OH}-($A_{NS,3S}$)(4.61/103.8 ppm)+G_{2OH}-(A_{NS})(4.60/104.6 ppm)+G_{2OH}-(A_{NAc})(4.51/105.0 ppm) ÷U-Ring C_1
ΔU_{2S}-C1	ΔU_{2S} C_1(5.50/100.1 ppm)÷U-Ring C_1
ΔU_{2OH}-C_1	ΔU_{2OH}(5.16/103.7 ppm)÷U-Ring C_1
I_{2S}-βRed-C_1	I_{2S}-βRed (4.99/94.6 ppm)÷U-Ring C_1
I_{2S}-αRed-C_1	I_{2S}-αRed (5.42/95.4 ppm)÷U-Ring C_1
Epo-I-C_2	Epo-I(3.73/54.2 ppm)÷A ring-C_2
GalA-C_5	GalA(4.66/74.4 ppm)÷Avg. A-ring
Linker-Xyl-C_1	Xyl (4.44/105.6 ppm)÷U-Ring C_1

Supplemental Table 8: Calculation of enoxaparin percent disaccharide composition in the USP enoxaparin standard based on 2D ^{1}H-^{13}C HSQC NMR H_1/C_1 cross-peak integrated volumes. The chemical shift value shown in parentheses are the signals integrated to obtain volumes.

Signals	**Integrated Crosspeaks ($^{1}H/^{13}C$ chemical shifts)**	**Int. Area**
A-Ring-C_1 (n=4)	A_{NS}-(G)(5.56/100.4 ppm)+$A_{NS,3S,6x}$(5.48/99.0 ppm)+[$A_{NS,6S}$-(I_{2S})+A_{NAc}-(G)](5.37/99.6 ppm)+$A_{NS,6x}$-(I)(5.34/98.2 ppm)+1,6-an.A(5.61/104.1 ppm)+1,6-an.M(5.56/103.7 ppm)+M_{NS}-αRed(5.39/95.6 ppm)+A_{NS}-αRed(5.44/93.9 ppm)+A_{NAc}-αRed(5.20/93.4 ppm)+A_{NS}-βRed(4.71/98.5 ppm)	59 ± 5
U/I/G-Ring-C_1 (n=4)	ΔU_{2S}(5.50/100.1 ppm)+ΔU_{2OH}(5.16/103.7 ppm)+I_{2S}-($A_{NS,6x}$)(5.22/102.0 ppm)+I_{2OH}-($A_{NS,6S}$)(5.00/104.8 ppm)+I_{2OH}-(A_{NS})(4.94/104.5 ppm)+ G_{2S}-(A_{NS})(4.73/102.9 ppm)+G_{2OH}-($A_{NS,3S}$)(4.61/103.8 ppm)+G_{2OH}-(A_{NS})(4.60/104.6 ppm)+G_{2OH}-(A_{NAc})(4.51/105.0 ppm)+I_{2S}-βRed (4.99/94.6 ppm)+I_{2S}-αRed (5.42/95.4 ppm)$_{2S}$-βRed (4.59/104.7 ppm)+I_{2S}-αRed (5.42/95.4 ppm)	61 ± 5

%Disaccharide	**Calculation used**
$A_{NS,6x}$-(I_{2S})+A_{NAc}-(G)	[$A_{NS,6x}$-(I_{2S})+A_{NAc}-(G)](5.37/99.6 ppm)÷A-Ring-C_1
$A_{NS,6x}$-(I_{2OH})	$A_{NS,6x}$-(I_{2OH})(5.34/98.2 ppm)÷A-Ring-C_1
$A_{NS,6x}$-(G)	$A_{NS,6x}$-(G)(5.56/100.4 ppm)÷A-Ring-C_1
$A_{NS,3S,6x}$-(x)	$A_{NS,3S,6x}$(5.48/99.0 ppm)÷A-Ring-C_1
I_{2S}-($A_{NS,6S}$)	I_{2S}-($A_{NS,6x}$)(5.22/102.0 ppm)÷U-Ring-C_1
I_{2OH}-($A_{NS,6S}$)	I_{2OH}-($A_{NS,6S}$)(5.00/104.8 ppm)÷U-Ring-C_1
I_{2OH}-($A_{NS,6OH}$)	I_{2OH}-($A_{NS,6OH}$)(4.94/104.5 ppm)÷U-Ring-C_1
G_{2S}-(A_{NS})	G_{2S}-(A_{NS})(4.73/102.9 ppm)÷U-Ring C_1
G_{2OH}-(A_{NS})	G_{2OH}-(A_{NS})(4.60/104.6 ppm)÷U-Ring-C_1
G_{2OH}-($A_{NS,3S}$)	G_{2OH}-($A_{NS,3S}$)(4.61/103.8 ppm)÷U-Ring-C_1
ΔU_{2S}-(A)	ΔU_{2S}(5.50/100.1 ppm)÷U-Ring-C_1
ΔU_{2OH}-(A)	ΔU_{2OH}(5.16/103.7 ppm)÷U-Ring-C_1

Supplemental Table 9: Comparison of integrated 2D-HSQC volumes acquired with different parameters on the same sample. The values are the integrated volume of each signal divided by the comparison spectrum. In the case of the signal to noise comparison all volumes were normalized to the volume obtained with the 2.7 h experiment. For the relaxation delay comparison all values were divided by the values obtained in the 10.9 h experiment.

Signal Integrated	Relaxation Delay Comparison (nt=32)	Signal-to-Noise Comparison		
Parameter Altered→	d1=12.6/1.5	nt=32/8	nt=16/8	nt=8/8
Experiment Time Ratio→	68.3/10.9 h	10.9/2.7 h	5.4/2.7 h	2.7/2.7 h
$A_{NAc,6x}$-C2	1.32	4.41	2.13	1.00
$A_{NS,3S,6x}$-C2	1.57	3.90	1.79	1.00
$A_{NS,6x}$-C2	1.30	3.79	1.97	1.00
$A_{NS,6OH}$-C6	1.13	3.74	1.93	1.00
$A_{NS,6S}$-C6	1.50	3.95	2.04	1.00
A_{NS}-αRed-C1	1.55	4.70	1.90	1.00
$A_{NS,6x}$-I_{2OH}-C1	1.56	4.14	2.02	1.00
$A_{NS,6S}$-I_{2S}+A_{NAc}-G-C1	1.62	3.92	2.03	1.00
A_{NS}-G-C1	1.38	4.70	2.27	1.00
$A_{NS,3S,6S}$-C1	1.49	4.13	2.01	1.00
I_{2S}-$A_{NS,6x}$-C1	1.36	3.98	2.04	1.00
I_{2OH}-$A_{NS,6S}$-C1	1.36	3.62	1.88	1.00
I_{2OH}-A_{NS}-C1	1.56	3.28	1.74	1.00
G_{2OH}-$A_{NS,3S,6S}$-C1	1.53	3.67	1.93	1.00
G_{2OH}-A_{NAc}-C1	1.55	4.29	2.17	1.00
G_{2OH}-A_{NS}-C1	1.53	3.88	2.01	1.00
A_{NAc}-Methyl	1.64	3.59	1.75	1.00
Xyl-Linker-C1	1.71	3.61	1.88	1.00
Mean (n=18)	1.48	3.96	1.97	
Standard Deviation	0.14	0.38	0.14	
%RSD	9.7%	9.7%	7.1%	