Supplementary Data

- Tri - all heavy: 2
- Tri - h1: 4
- Tri - h2: 6
- Tri - h3: 8
- Tetra - all heavy: 10
- Tetra - h1: 12
- Tetra - h2: 14
- Tetra - h3: 16
- Tetra - hAla: 18
- Tetra - h1h2: 20
- Tetra - h3Ala: 22
- Additional monomer hybrids: 24
- Tri-Tri - labeled: 25
- Tri-Tri - unlabeled: 27
- Tri-Tri - Donor all light: 29
- Tetra-Tri - labeled: 31
- Tetra-Tri - unlabeled: 33
- Tetra-Tri - Donor all light: 35
- Tri-Tetra - labeled: 37
- Tri-Tetra - unlabeled: 39
- Tri-Tetra - Donor all light: 41
- Tetra-Tetra - labeled: 43
- Tetra-Tetra - unlabeled: 45
- Tetra-Tetra - Donor all light: 47
Monomers

Supplementary Data 1.1: Tandem mass spectrometry analysis of the labeled disaccharide-tripeptide. The observed $m/z_{obs}$ and calculated $m/z_{calc}$ values of the parental ion, $[M+H]^+$, as determined in the absence of fragmentation (MS1), are indicated. The $m/z_{calc}$ value was used to select the ion for fragmentation. The difference between the $m/z_{obs}$ and $m/z_{calc}$ values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcN\textsuperscript{Red}), the acetyl group (Ac), and the D-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively. The peaks corresponding to the list of fragments appearing in the table are highlighted by orange dots in the mass spectrum. In the mass spectrum, peaks differing by the loss of H\textsubscript{2}O are connected by a dashed line. The tables only contain the $m/z$ values for the fragments containing H\textsubscript{2}O.

An interactive report of the MS\textsuperscript{2} analysis is available in Supplementary File F1.1.

| Precursor ion (MS1) | $m/z_{obs}$ | $m/z_{calc}$ | ppm |
|---------------------|-------------|--------------|-----|
| GlcN(-Ac)-GlcN\textsuperscript{Red}(-Ac)-Lac-Ala-Glu-DAP | 911.475 | 911.475 | 0.3 |

| Product ion | $m/z_{obs}$ | $m/z_{calc}$ | ppm | Intensity (a.u.) | Isotopologue |
|-------------|-------------|--------------|-----|-----------------|--------------|
| GlcN\textsuperscript{Red}(-Ac)-Lac-Ala-Glu-DAP | 699.371 | 699.371 | 0.7 | 7650 | all heavy |
| GlcN\textsuperscript{Red}(-Ac)-Lac-Ala-Glu | 500.257 | 500.259 | 2.7 | 570 | all heavy |
| Lac-Ala-Glu-DAP | 485.254 | 485.253 | -2.7 | 720 | all heavy |
| Ala-Glu-DAP | 410.220 | 410.221 | 3.7 | 3226 | all heavy |
| GlcN\textsuperscript{Red}(-Ac)-Lac-Ala | 365.201 | 365.202 | 2.5 | 4356 | all heavy |
| Glu-DAP | 335.177 | 335.177 | 0.3 | 9504 | all heavy |
| GlcN\textsuperscript{Red}(-Ac)-Lac | 290.158 | 290.158 | -1.0 | 1835 | all heavy |
| GlcN\textsuperscript{Red}(-Ac) | 215.127 | 215.127 | 0.5 | 229 | all heavy |
| GlcN(-Ac) | 213.110 | 213.111 | 3.7 | 108 | all heavy |
| DAP | 200.122 | 200.121 | -4.1 | 3273 | all heavy |
Supplementary Data 1.2: Tandem mass spectrometry analysis of the h1-type hybrid of the disaccharide-tripeptide. The molecule is fully unlabeled except for one of the two glucosamine moieties present in GlcNAc or MurNAc leading to the presence of two isotopomers. The observed \( m/z \) \( \text{obs} \) and calculated \( m/z \) \( \text{calc} \) values of the parental ion, \([M+H]^+\), as determined in the absence of fragmentation (MS\(^1\)), are indicated. The \( m/z \) \( \text{calc} \) value was used to select the ion for fragmentation. The difference between the \( m/z \) \( \text{obs} \) and \( m/z \) \( \text{calc} \) values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcN\(^\text{Red}\)), the acetyl group (Ac), and the D-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively. The fragmentation led to two series of fragments specific of the isotopomers containing the unlabeled glucosamine moiety in the GlcNAc (h1G) or MurNAc\(^\text{Red}\) (h1M) residues, respectively (discriminatory fragments). The fragments specific of h1G and of h1M are highlighted by orange and green dots in the mass spectrum. The other fragments are common to the fragmentation patterns of h1G and of h1M. The corresponding peaks are highlighted by blue dots in the mass spectrum. The mass spectrum indicates the presence of both isotopomers as expected from the recycling and synthesis pathways since UDP-MurNAc exclusively derives from UDP-GlcNAc. In the mass spectrum, peaks differing by the loss of \( \text{H}_2\text{O} \) are connected by a dashed line. The tables only contain the \( m/z \) values for the fragments containing \( \text{H}_2\text{O} \). An interactive report of the MS\(^2\) analysis is available in Supplementary File F1.2.

| Precursor Ion (MS\(^1\)) | \( m/z \) \( \text{obs} \) | \( m/z \) \( \text{calc} \) | ppm | Intensity (a.u.) | Isotopomer |
|--------------------------|-----------------------|---------------------|-----|----------------|-----------|
| GlcN\(^\text{Ac}\)GlcN\(^\text{Red}\)(-Ac)-Lac-Ala-Glu-DAP | 878.392 | 878.396 | -4.0 | | |
| GlcN\(^\text{Ac}\)GlcN\(^\text{Red}\)(-Ac)-Lac-Ala-Glu-DAP | 878.392 | 878.396 | -4.0 | | |
| Discriminatory product ion | \( m/z \) \( \text{obs} \) | \( m/z \) \( \text{calc} \) | ppm | Intensity (a.u.) | Isotopomer |
| GlcN\(^\text{Red}\)-(Ac)-Lac-Ala-Glu-DAP | 675.318 | 675.316 | -2.3 | 10553 | h1M |
| GlcN\(^\text{Red}\)-(Ac)-Lac-Ala-Glu-DAP | 668.299 | 668.299 | 0.6 | 8694 | h1G |
| GlcN\(^\text{Red}\)-(Ac)-Lac | 485.221 | 485.221 | 0.3 | 952 | h1M |
| GlcN\(^\text{Red}\)-(Ac)-Lac-Ala-Glu | 478.202 | 478.204 | 3.4 | 662 | h1G |
| GlcN\(^\text{Red}\)-(Ac)-Lac-Ala | 356.177 | 356.178 | 3.6 | 7236 | h1M |
| GlcN\(^\text{Red}\)-(Ac)-Lac-Ala-Glu | 349.161 | 349.161 | 1.1 | 6203 | h1G |
| GlcN\(^\text{Red}\)-(Ac)-Lac-Ala | 285.140 | 285.141 | 4.9 | 3223 | h1M |
| GlcN\(^\text{Red}\)-(Ac)-Lac-Ala-Glu | 278.123 | 278.124 | 4.0 | 3059 | h1G |
| GlcN\(^\text{Red}\)-(Ac) | 213.118 | 213.120 | 8.2 | 247 | h1M |
| GlcN\(^\text{Red}\)-(Ac) | 211.106 | 211.104 | -6.8 | 238 | h1G |
| GlcN\(^\text{Red}\)-(Ac) | 206.103 | 206.103 | -0.5 | 148 | h1G |
| GlcN\(^\text{Red}\)-(Ac)DAP | 204.084 | 204.087 | 15.1 | 419 | h1M |
| Common product ion | \( m/z \) \( \text{obs} \) | \( m/z \) \( \text{calc} \) | ppm | Intensity (a.u.) |
| GlcN\(^\text{Red}\)-(Ac)-GlcN\(^\text{Red}\)(-Ac)-Lac-Ala-Glu-DAP | 878.397 | 878.396 | -2.1 | 178 |
| GlcN\(^\text{Red}\)-(Ac)-GlcN\(^\text{Red}\)(-Ac)-Lac-Ala-Glu | 559.257 | 559.258 | 0.8 | 112 |
| Lac-Ala-Glu-DAP | 463.203 | 463.204 | 1.8 | 613 |
| Ala-Glu-DAP | 391.182 | 391.183 | 2.0 | 9574 |
| Glu-DAP | 320.146 | 320.146 | 0.3 | 26374 |
| Ala-Glu | 201.085 | 201.086 | 13.5 | 227 |
| DAP | 191.103 | 191.103 | 0.6 | 6654 |
| Lac-Ala | 144.068 | 144.066 | -10.4 | 154 |
Supplementary Data 1.3: Tandem mass spectrometry analysis of the h2-type hybrid of the disaccharide-tripeptide. The observed $m/z_{\text{obs}}$ and calculated $m/z_{\text{calc}}$ values of the parental ion, $[\text{M+H}]^+$, as determined in the absence of fragmentation (MS1), are indicated. The $m/z_{\text{calc}}$ value was used to select the ion for fragmentation. The difference between the $m/z_{\text{obs}}$ and $m/z_{\text{calc}}$ values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcN\textsuperscript{Red}), the acetyl group (Ac), and the D-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively. The peaks corresponding to the list of fragments appearing in the table are highlighted by orange dots in the mass spectrum. In the mass spectrum, peaks differing by the loss of $\text{H}_2\text{O}$ are connected by a dashed line. The tables only contain the $m/z$ values for the fragments containing $\text{H}_2\text{O}$.

An interactive report of the MS\textsuperscript{2} analysis is available in Supplementary File F1.3.

| Precursor ion (MS1) | $m/z_{\text{obs}}$ | $m/z_{\text{calc}}$ | ppm |
|---------------------|---------------------|---------------------|-----|
| GlcN(-Ac)-GlcN\textsuperscript{Red}(-Ac)-Lac-Ala-Glu-DAP | 890.412 | 890.417 | -5.7 |

| Product ion | $m/z_{\text{obs}}$ | $m/z_{\text{calc}}$ | ppm | Intensity (a.u.) | Isotopologue |
|-------------|---------------------|---------------------|-----|------------------|--------------|
| GlcN\textsuperscript{Red}(-Ac)-Lac-Ala-Glu-DAP | 687.336 | 687.338 | 2.2 | 7465 | h2 |
| GlcN\textsuperscript{Red}(-Ac)-Lac-Ala-Glu | 488.223 | 488.225 | 3.1 | 404 | h2 |
| Lac-Ala-Glu-DAP | 482.239 | 482.242 | 7.1 | 296 | h2 |
| Ala-Glu-DAP | 410.219 | 410.221 | 5.5 | 3431 | h2 |
| GlcN\textsuperscript{Red}(-Ac)-Lac-Ala | 353.167 | 353.168 | 4.7 | 4771 | h2 |
| Glu-DAP | 335.178 | 335.177 | -2.4 | 9561 | h2 |
| GlcN\textsuperscript{Red}(-Ac)-Lac | 278.123 | 278.124 | 2.9 | 1727 | h2 |
| GlcN(-Ac) | 204.086 | 204.087 | 5.9 | 190 | h2 |
| DAP | 200.120 | 200.121 | 4.2 | 2800 | h2 |
Supplementary Data 1.4: Tandem mass spectrometry analysis of the h3-type hybrid of the disaccharide-tripeptide. The observed \( m/z_{\text{obs}} \) and calculated \( m/z_{\text{calc}} \) values of the parental ion, [M+H]⁺, as determined in the absence of fragmentation (MS1), are indicated. The \( m/z_{\text{calc}} \) value was used to select the ion for fragmentation. The difference between the \( m/z_{\text{obs}} \) and \( m/z_{\text{calc}} \) values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcN\text{Red}), the acetyl group (Ac), and the D-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively. The peaks corresponding to the list of fragments appearing in the table are highlighted by orange dots in the mass spectrum.

In the mass spectrum, peaks differing by the loss of H₂O are connected by a dashed line. The tables only contain the \( m/z \) values for the fragments containing H₂O. The presence of the h3 isotopomer is expected since cells of *E. coli* contain the main PG precursor, UDP-MurNAc-pentapeptide in considerable amounts (about 2% compared to the total amount of dissaccharide peptides in the cell wall). An interactive report of the MS² analysis is available in Supplementary File F1.4.

| Precursor ion (MS1) | \( m/z_{\text{obs}} \) | \( m/z_{\text{calc}} \) | ppm | Intensity (a.u.) | Isotopomer |
|---------------------|------------------------|------------------------|-----|-----------------|------------|
| GlcN(-Ac)-GlcN\text{Red}(-Ac)-Lac-Ala-Glu-DAP | 902.447 | 902.451 | -3.8 |                |            |

| Product ion | \( m/z_{\text{obs}} \) | \( m/z_{\text{calc}} \) | ppm | Intensity (a.u.) | Isotopomer |
|-------------|------------------------|------------------------|-----|-----------------|------------|
| GlcN\text{Red}(-Ac)-Lac-Ala-Glu-DAP | 699.372 | 699.371 | -0.5 | 4005 | h3 |
| GlcN\text{Red}(-Ac)-Lac-Ala-Glu | 500.259 | 500.259 | -0.8 | 329 | h3 |
| Lac-Ala-Glu-DAP | 485.251 | 485.253 | 3.1 | 308 | h3 |
| Ala-Glu-DAP | 410.219 | 410.221 | 5.1 | 1961 | h3 |
| GlcN\text{Red}(-Ac)-Lac | 365.199 | 365.202 | 8.5 | 2535 | h3 |
| Glu-DAP | 335.178 | 335.177 | -2.9 | 5470 | h3 |
| GlcN\text{Red}(-Ac)-Lac | 290.158 | 290.158 | 0.2 | 1047 | h3 |
| GlcN(-Ac) | 204.088 | 204.087 | -2.6 | 132 | h3 |
| DAP | 200.120 | 200.121 | 3.8 | 1331 | h3 |
Supplementary Data 2.1: Supplementary Data D2.1. Tandem mass spectrometry analysis of the labeled disaccharide-tetrapeptide.

The observed \( m/z_{\text{obs}} \) and calculated \( m/z_{\text{calc}} \) values of the parental ion, \([\text{M+H}]^+\), as determined in the absence of fragmentation (MS1), are indicated. The \( m/z_{\text{calc}} \) value was used to select the ion for fragmentation. The difference between the \( m/z_{\text{obs}} \) and \( m/z_{\text{calc}} \) values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcN\text{Red}), the acetyl group (Ac), and the \( \delta \)-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively. The peaks corresponding to the list of fragments appearing in the table are highlighted by orange dots in the mass spectrum. In the mass spectrum, peaks differing by the loss of \( \text{H}_2\text{O} \) are connected by a dashed line. The tables only contain the \( m/z \) values for the fragments containing \( \text{H}_2\text{O} \).

An interactive report of the MS2 analysis is available in Supplementary File F2.1.

| Precursor ion (MS1)                                      | \( m/z_{\text{obs}} \) | \( m/z_{\text{calc}} \) | ppm   | Intensity (a.u.) | Isotopologue  |
|---------------------------------------------------------|------------------------|--------------------------|-------|------------------|---------------|
| GlcN(-Ac)-GlcN\text{Red}(-Ac)-Lac-Ala-Glu-DAP-Ala       | 986.516                | 986.519                  | -3.1  |                  |               |

| Product ion                                             | \( m/z_{\text{obs}} \) | \( m/z_{\text{calc}} \) | ppm   | Intensity (a.u.) | Isotopologue  |
|---------------------------------------------------------|------------------------|--------------------------|-------|------------------|---------------|
| GlcN\text{Red}(-Ac)-Lac-Ala-Glu-DAP-Ala                 | 774.423                | 774.416                  | -9.3  | 5487             | all heavy     |
| GlcN\text{Red}(-Ac)-Lac-Ala-Glu-DAP                     | 681.364                | 681.361                  | -5.1  | 494              | all heavy     |
| Lac-Ala-Glu-DAP-Ala                                     | 560.296                | 560.297                  | 0.9   | 119              | all heavy     |
| GlcN\text{Red}(-Ac)-Lac-Ala-Glu                        | 500.259                | 500.259                  | 0.0   | 194              | all heavy     |
| Ala-Glu-DAP-Ala                                         | 485.269                | 485.266                  | -6.8  | 1147             | all heavy     |
| Glu-DAP-Ala                                             | 410.223                | 410.221                  | -3.9  | 3963             | all heavy     |
| Ala-Glu-DAP                                             | 392.213                | 392.211                  | -5.6  | 1512             | all heavy     |
| GlcN\text{Red}(-Ac)-Lac-Ala                             | 365.204                | 365.202                  | -5.1  | 2434             | all heavy     |
| Glu-DAP                                                 | 317.169                | 317.167                  | -8.5  | 3861             | all heavy     |
| GlcN\text{Red}(-Ac)-Lac                                 | 290.160                | 290.158                  | -7.3  | 414              | all heavy     |
| DAP-Ala                                                 | 275.167                | 275.165                  | -6.2  | 3667             | all heavy     |
| DAP                                                     | 182.112                | 182.110                  | -8.1  | 611              | all heavy     |
Supplementary Data 2.2: Tandem mass spectrometry analysis of the h1-type hybrid of the disaccharide-tetrapeptide. The molecule is fully unlabeled except for one of the two glucosamine moieties present in GlcNAc or MurNac leading to the presence of two isotopomers. The observed m/z values and calculated m/z values of the parental ion, [M+H]+, as determined in the absence of fragmentation (MS1), are indicated. The m/z value was used to select the ion for fragmentation. The difference between the m/z values and m/z values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNac residue is indicated in three moieties, reduced glucosamine (GlcNRed), the acetyl group (Ac), and the D-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively. The fragmentation led to two series of fragments specific of the isotopomers containing the unlabeled glucosamine moiety in the GlcNAc (h1G) or MurNacRed (h1M) residues, respectively (discriminatory fragments). The fragments specific of h1G and of h1M are highlighted by orange and green dots in the mass spectrum. The other fragments are common to the fragmentation patterns of h1G and of h1M. The corresponding peaks are highlighted by blue dots in the mass spectrum. The mass spectrum indicates the presence of both isotopomers as expected from the recycling and synthesis pathways since UDP-MurNAc exclusively derives from UDP-GlcNAc. In the mass spectrum, peaks differing by the loss of H2O are connected by a dashed line. The tables only contain the m/z values for the fragments containing H2O. An interactive report of the MS2 analysis is available in Supplementary File F2.2.

| Precursor ion (MS1) | m/zobs | m/zcalc | ppm  | Intensity (a.u.) | Isotopologue |
|---------------------|--------|---------|------|-----------------|--------------|
| GlcN(-Ac)-GlcNRed(-Ac)-Lac-Ala-Glu-DAP-Ala | 949.429 | 949.433 | -4.0 | 5188 | h1M |
| GlcN(-Ac)-GlcNRed(-Ac)-Lac-Ala-Glu-DAP-Ala | 949.429 | 949.433 | -4.0 | 5188 | h1M |
| Discriminatory product ions | m/zobs | m/zcalc | ppm  | Intensity (a.u.) | Isotopologue |
| GlcNRed(-Ac)-Lac-Ala-Glu-DAP-Ala | 746.351 | 746.353 | 3.3 | 5188 | h1M |
| GlcNRed(-Ac)-Lac-Ala-Glu-DAP-Ala | 739.333 | 739.336 | 4.7 | 3690 | h1G |
| GlcNRed(-Ac)-Lac-Ala-Glu-DAP | 657.302 | 657.306 | 5.2 | 477 | h1M |
| GlcNRed(-Ac)-Lac-Ala-Glu-DAP | 650.287 | 650.288 | 2.7 | 427 | h1G |
| GlcNRed(-Ac)-Lac-Ala-Glu | 485.216 | 485.221 | 10.3 | 417 | h1M |
| GlcNRed(-Ac)-Lac-Ala-Glu | 478.202 | 478.204 | 3.3 | 210 | h1G |
| GlcNRed(-Ac)-Lac | 356.177 | 356.178 | 3.6 | 1850 | h1M |
| GlcNRed(-Ac)-Lac | 349.158 | 349.161 | 8.1 | 2036 | h1G |
| GlcNRed(-Ac)-Lac-Ala | 285.140 | 285.141 | 4.9 | 586 | h1M |
| GlcNRed(-Ac)-Lac | 278.123 | 278.124 | 4.0 | 622 | h1G |
| GlcN(-Ac) | 204.086 | 204.087 | 7.2 | 137 | h1M |
| Common product ion | m/zobs | m/zcalc | ppm  | Intensity (a.u.) |
| GlcN(-Ac)-GlcNRed(-Ac)-Lac-Ala-Glu-DAP-Ala | 949.433 | 949.433 | 0.1 | 241 |
| GlcN(-Ac)-GlcNRed(-Ac)-Lac-Ala-Glu-DAP-Ala | 462.217 | 462.220 | 6.1 | 1977 |
| Ala-Glu-DAP-Ala | 391.180 | 391.183 | 6.2 | 7517 |
| Ala-Glu-DAP | 373.172 | 373.172 | 1.7 | 2756 |
| DAP-Ala | 302.135 | 302.135 | 0.9 | 7122 |
| Glu-DAP | 262.138 | 262.140 | 9.0 | 5564 |
| DAP | 173.093 | 173.093 | 0.3 | 796 |
Supplementary Data 2.3: Tandem mass spectrometry analysis of the h2-type hybrid of the disaccharide-tetrapeptide. The observed $m/z_{obs}$ and calculated $m/z_{calc}$ values of the parental ion, [M+H]$^+$, as determined in the absence of fragmentation (MS1), are indicated. The $m/z_{calc}$ value was used to select the ion for fragmentation. The difference between the $m/z_{obs}$ and $m/z_{calc}$ values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcN$^{\text{Red}}$), the acetyl group (Ac), and the d-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively. The peaks corresponding to the list of fragments appearing in the table are highlighted by orange dots in the mass spectrum. In the mass spectrum, peaks differing by the loss of H$_2$O are connected by a dashed line. The tables only contain the $m/z$ values for the fragments containing H$_2$O.

An interactive report of the MS$^2$ analysis is available in Supplementary File F2.3.

| Precursor ion (MS1) | $m/z_{obs}$ | $m/z_{calc}$ | ppm | Intensity (a.u.) | Isotopologue |
|---------------------|-------------|--------------|-----|-----------------|--------------|
| GlcN(-Ac)-GlcN$^{\text{Red}}$(-Ac)-Lac-Ala-Glu-DAP-Ala | 961.451 | 961.454 | -2.7 | 250 | h2 |
| GlcN$^{\text{Red}}$(-Ac)-Lac-Ala-Glu-DAP-Ala | 758.374 | 758.375 | 1.0 | 15348 | h2 |
| GlcN$^{\text{Red}}$(-Ac)-Lac-Ala-Glu-DAP | 669.327 | 669.327 | -0.8 | 1466 | h2 |
| GlcN(-Ac)-GlcN$^{\text{Red}}$(-Ac)-Lac-Ala | 556.244 | 556.248 | 7.1 | 191 | h2 |
| Lac-Ala-Glu-DAP-Ala | 553.280 | 553.280 | -0.4 | 302 | h2 |
| GlcN$^{\text{Red}}$(-Ac)-Lac-Ala-Glu | 488.224 | 488.225 | 1.8 | 1041 | h2 |
| Ala-Glu-DAP-Ala | 481.259 | 481.258 | -1.0 | 3833 | h2 |
| Lac-Ala-Glu-DAP | 464.235 | 464.232 | -6.3 | 133 | h2 |
| Glu-DAP-Ala | 406.212 | 406.214 | 6.4 | 13380 | h2 |
| Ala-Glu-DAP | 392.208 | 392.211 | 6.8 | 4340 | h2 |
| GlcN$^{\text{Red}}$(-Ac)-Lac-Ala | 353.167 | 353.168 | 3.4 | 6673 | h2 |
| Glu-DAP | 317.165 | 317.167 | 3.8 | 12830 | h2 |
| GlcN$^{\text{Red}}$(-Ac)-Lac | 278.122 | 278.124 | 7.6 | 1991 | h2 |
| DAP-Ala | 271.156 | 271.158 | 7.2 | 10798 | h2 |
| GlcN$^{\text{Red}}$(-Ac) | 206.103 | 206.103 | 2.8 | 163 | h2 |
| GlcN(-Ac) | 204.085 | 204.087 | 12.7 | 229 | h2 |
| DAP | 182.108 | 182.110 | 9.5 | 1297 | h2 |
**Supplementary Data 2.4: Tandem mass spectrometry analysis of the h3-type hybrid of the disaccharide-tetrapeptide.** The observed $m/z_{\text{obs}}$ and calculated $m/z_{\text{calc}}$ values of the parental ion, [M+H]$,^+$, as determined in the absence of fragmentation (MS1), are indicated. The $m/z_{\text{calc}}$ value was used to select the ion for fragmentation. The difference between the $m/z_{\text{obs}}$ and $m/z_{\text{calc}}$ values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcN$^{\text{Red}}$), the acetyl group (Ac), and the D-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively. The peaks corresponding to the list of fragments appearing in the table are highlighted by orange dots in the mass spectrum. In the mass spectrum, peaks differing by the loss of $\text{H}_2\text{O}$ are connected by a dashed line. The tables only contain the $m/z$ values for the fragments containing $\text{H}_2\text{O}$. The presence of the h3 isotopomer is expected since cells of *E. coli* contain the main PG precursor, UDP-MurNAc-pentapeptide in considerable amounts (about 2% compared to the total amount of disaccharide peptides in the cell wall). An interactive report of the MS$^2$ analysis is available in Supplementary File F2.4.

| Precursor ion (MS1) | $m/z_{\text{obs}}$ | $m/z_{\text{calc}}$ | ppm | Intensity (a.u.) | Isotopologue |
|---------------------|---------------------|---------------------|-----|-----------------|--------------|
| GlcN(-Ac)-GlcN$^{\text{Red}}$(-Ac)-Lac-Ala-Glu-DAP-Ala | 977.490 | 977.490 | -5.0 | | |

| Product ion | $m/z_{\text{obs}}$ | $m/z_{\text{calc}}$ | ppm | Intensity (a.u.) | Isotopologue |
|-------------|---------------------|---------------------|-----|-----------------|--------------|
| GlcN$^{\text{Red}}$(-Ac)-Lac-Ala-Glu-DAP-Ala | 774.413 | 774.416 | 3.0 | 3734 | h3 |
| GlcN$^{\text{Red}}$(-Ac)-Lac-Ala-Glu-DAP | 681.368 | 681.361 | -9.8 | 306 | h3 |
| GlcN$^{\text{Red}}$(-Ac)-Lac-Ala-Glu | 500.260 | 500.259 | -2.0 | 259 | h3 |
| Ala-Glu-DAP-Ala | 485.265 | 485.266 | 1.6 | 834 | h3 |
| Glu-DAP-Ala | 410.220 | 410.221 | 3.9 | 2916 | h3 |
| Ala-Glu-DAP | 392.208 | 392.211 | 6.4 | 1481 | h3 |
| GlcN$^{\text{Red}}$(-Ac)-Lac-Ala | 365.199 | 365.202 | 7.3 | 1567 | h3 |
| Glu-DAP | 317.165 | 317.167 | 3.4 | 3046 | h3 |
| GlcN$^{\text{Red}}$(-Ac)-Lac | 290.156 | 290.158 | 7.5 | 508 | h3 |
| DAP-Ala | 275.163 | 275.165 | 6.7 | 2174 | h3 |
| DAP | 182.110 | 182.110 | 0.1 | 354 | h3 |
**Additional Monomer hybrids**

**Supplementary Data 2.5: Tandem mass spectrometry analysis of the hAla-type hybrid of the disaccharide-tetrapeptide.** The observed $m/z_{obs}$ and calculated $m/z_{calc}$ values of the parental ion, $[\text{M+H}]^+$, as determined in the absence of fragmentation (MS1), are indicated. The $m/z_{calc}$ value was used to select the ion for fragmentation. The difference between the $m/z_{obs}$ and $m/z_{calc}$ values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcN$^{\text{Red}}$), the acetyl group (Ac), and the D-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively. The peaks corresponding to the list of fragments appearing in the table are highlighted by orange dots in the mass spectrum. In the mass spectrum, peaks differing by the loss of H$_2$O are connected by a dashed line. The tables only contain the $m/z$ values for the fragments containing H$_2$O.

An interactive report of the MS2 analysis is available in Supplementary File F2.5.

| Parent (MS1) | $m/z_{obs}$ | $m/z_{calc}$ | ppm | m/z | Intensity (a.u.) | Isotopologue |
|--------------|-------------|--------------|-----|-----|-----------------|--------------|
| GlcN-(Ac)-GlcN$^{\text{Red}}$-(Ac)-Lac-Ala-Glu-DAP-Ala | 946.420 | 946.423 | -2.9 | hAla4 |

| Fragment | $m/z_{obs}$ | $m/z_{calc}$ | ppm | Intensity (a.u.) | Isotopologue |
|----------|-------------|--------------|-----|-----------------|--------------|
| GlcN-(Ac)-GlcN$^{\text{Red}}$-(Ac)-Lac-Ala-Glu-DAP-Ala | 946.414 | 946.423 | 8.8 | 227 | hAla4 |
| GlcN$^{\text{Red}}$-(Ac)-Lac-Ala-Glu-DAP-Ala | 743.340 | 743.343 | 4.3 | 1657 | hAla4 |
| Ala-Glu-DAP-Ala | 466.232 | 466.227 | -9.5 | 194 | hAla4 |
| Glu-DAP-Ala | 395.191 | 395.190 | -3.2 | 616 | hAla4 |
| Ala-Glu-DAP | 373.172 | 373.172 | 0.3 | 84 | hAla4 |
| GlcN$^{\text{Red}}$-(Ac)-Lac-Ala | 349.162 | 349.161 | -2.2 | 131 | hAla4 |
| Glu-DAP | 302.133 | 302.135 | 7.2 | 279 | hAla4 |
| GlcN$^{\text{Red}}$-(Ac)-Lac | 278.125 | 278.124 | -2.5 | 60 | hAla4 |
| DAP-Ala | 266.146 | 266.147 | 4.3 | 73 | hAla4 |
| DAP | 173.091 | 173.093 | 10.9 | 150 | hAla4 |
Supplementary Data 2.6: Tandem mass spectrometry analysis of the h1h2-type hybrid of the disaccharide-tetrapeptide. The molecule is fully unlabeled except for one of the two glucosamine moieties present in GlcNAc or MurNAc leading to the presence of two isotopomers.

The observed \( m/z_{\text{obs}} \) and calculated \( m/z_{\text{calc}} \) values of the parental ion, \([M+H]^+\), as determined in the absence of fragmentation (MS\(^1\)), are indicated. The \( m/z_{\text{cal}} \) value was used to select the ion for fragmentation. The difference between the \( m/z_{\text{obs}} \) and \( m/z_{\text{cal}} \) values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcN\text{Red})



| Precursor ion (MS1) | \( m/z_{\text{obs}} \) | \( m/z_{\text{calc}} \) | ppm | Intensity (a.u.) | Isotopologue |
|---------------------|-------------------------|-------------------------|-----|------------------|--------------|
| GlcN(-Ac)-GlcN\text{Red}(-Ac)-Lac-Ala-Glu-DAP-Ala | 968.471 | 968.471 | 0.2 | 469 | h1Mh2 |
| GlcN(-Ac)-GlcN\text{Red}(-Ac)-Lac-Ala-Glu-DAP-Ala | 968.471 | 968.471 | 0.2 | 309 | h1Gh2 |

| Discriminatory product ions | \( m/z_{\text{obs}} \) | \( m/z_{\text{calc}} \) | ppm | Intensity (a.u.) |
|-----------------------------|-------------------------|-------------------------|-----|-----------------|
| GlcN\text{Red}(-Ac)-Lac-Ala-Glu-DAP-Ala | 765.390 | 765.392 | 2.2 | 469 |
| GlcN\text{Red}(-Ac)-Lac-Ala-Glu-DAP-Ala | 758.370 | 758.375 | 6.2 | 309 |

| Common product ions | \( m/z_{\text{obs}} \) | \( m/z_{\text{calc}} \) | ppm | Intensity (a.u.) |
|---------------------|-------------------------|-------------------------|-----|-----------------|
| Glu-DAP-Ala | 406.212 | 406.214 | 5.8 | 151 |
| Glu-DAP | 317.163 | 317.167 | 12.6 | 121 |
| DAP | 182.108 | 182.110 | 11.6 | 60 |

An interactive report of the MS2 analysis is available in Supplementary File F2.6.
Supplementary Data 2.7: Tandem mass spectrometry analysis of the h3Ala-type hybrid of the disaccharide-tetrapeptide. The observed \( m/z_{\text{obs}} \) and calculated \( m/z_{\text{calc}} \) values of the parent ion, [M+H]\(^+\), as determined in the absence of fragmentation (MS1), are indicated. The \( m/z_{\text{calc}} \) value was used to select the ion for fragmentation. The difference between the \( m/z_{\text{obs}} \) and \( m/z_{\text{calc}} \) values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcN\(^{\text{Red}}\)), the acetyl group (Ac), and the D-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively. The peaks corresponding to the list of fragments appearing in the table are highlighted by orange dots in the mass spectrum. In the mass spectrum, peaks differing by the loss of H\(_2\)O are connected by a dashed line. The tables only contain the \( m/z \) values for the fragments containing H\(_2\)O.

An interactive report of the MS2 analysis is available in Supplementary File F2.7.

| Precursor Ion (MS1) | \( m/z_{\text{obs}} \) | \( m/z_{\text{calc}} \) | ppm | Intensity (a.u.) |
|---------------------|-------------------------|-------------------------|-----|------------------|
| GlcN(-Ac)-GlcN\(^{\text{Red}}\)(-Ac)-Lac-Ala-Glu-DAP-Ala | 973.484 | 973.488 | -3.9 |                |
| Product ion | \( m/z_{\text{obs}} \) | \( m/z_{\text{calc}} \) | ppm | Intensity (a.u.) |
| GlcN(-Ac)-GlcN\(^{\text{Red}}\)(-Ac)-Lac-Ala-Glu-DAP-Ala | 973.492 | 973.488 | -3.7 | 161 |
| GlcN\(^{\text{Red}}\)(-Ac)-Lac-Ala-Glu-DAP-Ala | 770.407 | 770.409 | 2.1 | 2018 |
| GlcN\(^{\text{Red}}\)(-Ac)-Lac-Ala-Glu-DAP | 681.362 | 681.361 | -1.4 | 91 |
| GlcN\(^{\text{Red}}\)(-Ac)-Lac-Ala-Glu | 500.260 | 500.259 | -3.4 | 65 |
| Ala-Glu-DAP-Ala | 481.254 | 481.258 | 9.6 | 113 |
| Glu-DAP-Ala | 406.215 | 406.214 | -2.1 | 544 |
| Ala-Glu-DAP | 392.207 | 392.211 | 10.6 | 138 |
| GlcN\(^{\text{Red}}\)(-Ac)-Lac-Ala | 365.201 | 365.202 | 2.0 | 332 |
| Glu-DAP | 317.167 | 317.167 | -1.6 | 259 |
| GlcN\(^{\text{Red}}\)(-Ac)-Lac | 290.158 | 290.158 | -0.3 | 62 |
| DAP-Ala | 271.155 | 271.158 | 11.1 | 111 |
| DAP | 182.108 | 182.110 | 12.8 | 20 |
Supplementary Data 2.8: Timecourses and structures of additional monomer hybrids. (A) Structure and timecourse of the h1h2 hybrid of the disaccharide tripeptide monomer combining a recycled tripeptide stem (h2) with a recycled glucosamine moiety (h1). (B) Additional hybrids of the disaccharide tetrapeptide comprise hAla (labeled C-terminal D-Ala\(^4\)), h1h2 (see (A)) and h3Ala (neo-synthesized GlcNAc and C-terminal D-Ala\(^4\)). Since disaccharide tripeptides are issued from disaccharide tetrapeptides by removal of the C-terminal D-Ala\(^4\), hAla and h3Ala are not detected for the tripeptide as these are converted into the uniformly unlabeled and h3 hybrid of the tripeptide, respectively. For structural characterization of the hAla, h1h2, and h3Ala hybrids see Supplementary Data above.
Supplementary Data 3.1: Tandem mass spectrometry analysis of the uniformly labeled Tri(3→3)Tri dimer. The observed \(m/z\) \(_{\text{obs}}\) and calculated \(m/z\) \(_{\text{calc}}\) values of the parental ion, [M+2H]\(^{2+}\), as determined in the absence of fragmentation (MS1), are indicated. The \(m/z\) \(_{\text{calc}}\) value was used to select the ion for fragmentation. The difference between the \(m/z\) \(_{\text{obs}}\) and \(m/z\) \(_{\text{calc}}\) values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcN\(^{\text{Red}}\)), the acetyl group (Ac), and the D-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively. The peaks corresponding to the list of fragments appearing in the table are highlighted by orange dots in the mass spectrum. In the mass spectrum, peaks differing by the loss of \(\text{H}_2\text{O}\) are connected by a dashed line. The tables only contain the \(m/z\) values for the fragments containing \(\text{H}_2\text{O}\). The arrows indicate the position of the 3→3 and 4→3 crosslinks. Residues in the acceptor are underlined.

An interactive report of the MS2 analysis is available in Supplementary File F3.1.

| Precursor ion (MS1) | \(m/z_{\text{obs}}\) [M+2H]\(^{2+}\) | \(m/z_{\text{calc}}\) [M+2H]\(^{2+}\) | ppm | Intensity (a.u.) |
|---------------------|-----------------|-----------------|-----|-----------------|
| GlcN(Ac)-GlcN\(^{\text{Red}}\)(Ac)-Lac-Ala-Glu-DAP\(\rightarrow\)DAP-Glu-Ala-Lac-GlcN\(^{\text{Red}}\)(Ac)-GlcN(Ac) | 902.464 | 902.469 | -5.9 | 52 |
| GlcN\(^{\text{Red}}\)(Ac)-Lac-Ala-Glu-DAP\(\rightarrow\)DAP-Glu-Ala-Lac-GlcN\(^{\text{Red}}\)(Ac) | 1591.833 | 1591.828 | -3.4 | 709 |
| GlcN\(^{\text{Red}}\)(Ac)-Lac-Ala-Glu-DAP\(\rightarrow\)DAP-Glu-Ala | 1379.721 | 1379.724 | 2.3 | 53 |
| GlcN\(^{\text{Red}}\)(Ac)-Lac-Ala-Glu-DAP\(\rightarrow\)DAP-Glu | 1090.570 | 1090.574 | 3.7 | 133 |
| DAP-Glu-Ala-Lac-GlcN\(^{\text{Red}}\)(Ac) | 699.371 | 699.371 | 1.1 | 71 |
| GlcN\(^{\text{Red}}\)(Ac)-Lac-Ala-Glu-DAP | 681.356 | 681.361 | 6.9 | 286 |
| GlcN\(^{\text{Red}}\)(Ac)-Lac-Ala | 365.200 | 365.202 | 5.6 | 77 |
| GlcN(Ac) | 213.109 | 213.111 | 7.8 | 860 |
Supplementary Data 3.2: Tandem mass spectrometry analysis of the uniformly unlabeled Tri(3→3)Tri dimer. The observed \( m/z_{\text{obs}} \) and calculated \( m/z_{\text{calc}} \) values of the parental ion, \([\text{M}+2\text{H}]^{2+}\), as determined in the absence of fragmentation (MS1), are indicated. The \( m/z_{\text{calc}} \) value was used to select the ion for fragmentation. The difference between the \( m/z_{\text{obs}} \) and \( m/z_{\text{calc}} \) values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcN\text{Red}), the acetyl group (Ac), and the D-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively. The peaks corresponding to the list of fragments appearing in the table are highlighted by orange dots in the mass spectrum.

In the mass spectrum, peaks differing by the loss of H\(_2\)O are connected by a dashed line. The tables only contain the \( m/z \) values for the fragments containing H\(_2\)O.

The arrows indicate the position of the 3→3 and 4→3 crosslinks. Residues in the acceptor are underlined.

An interactive report of the MS2 analysis is available in Supplementary File F3.2.

| Precursor ion (MS1) | \( m/z_{\text{obs}} \) \([\text{M}+2\text{H}]^{2+}\) | \( m/z_{\text{calc}} \) \([\text{M}+2\text{H}]^{2+}\) | ppm | Intensity (a.u.) |
|---------------------|-------------------|-------------------|-----|-----------------|
| GlcN\text{Ac})-GlcN\text{Ac})-Lac-Ala-Glu-DAP | 862.373 | 862.373 | 0.0 | 1 |

Product ion | \( m/z_{\text{obs}} \) \([\text{M}+\text{H}]^{+}\) | \( m/z_{\text{cal}} \) \([\text{M}+\text{H}]^{+}\) | ppm | Intensity (a.u.) |
|-------------|-------------------|-------------------|-----|-----------------|
| GlcN\text{Ac})-GlcN\text{Ac})-Lac-Ala-Glu-DAP | 1520.658 | 1520.659 | 0.9 | 528 |
| GlcN\text{Ac})-Lac-Ala-Glu-DAP | 1317.581 | 1317.580 | -1.3 | 14608 |
| GlcN\text{Ac})-Lac-Ala-Glu-DAP | 1172.522 | 1172.506 | -1.7 | 195 |
| GlcN\text{Ac})-Lac-Ala-Glu-DAP | 1112.491 | 1112.485 | -1.3 | 340 |
| GlcN\text{Ac})-Lac-Ala-Glu-DAP | 1040.466 | 1040.464 | -2.0 | 2012 |
| GlcN\text{Ac})-Lac-Ala-Glu-DAP | 969.428 | 969.426 | -1.3 | 10207 |
| Lac-Ala-Glu-DAP | 907.403 | 907.390 | -1.4 | 267 |
| Lac-Ala-Glu-DAP | 840.384 | 840.384 | -0.5 | 4637 |
| Lac-Ala-Glu-DAP | 835.377 | 835.369 | -1.0 | 208 |
| Lac-Ala-Glu-DAP | 764.336 | 764.331 | -0.5 | 517 |
| GlcN\text{Ac})-Lac-Ala-Glu-DAP | 763.357 | 763.347 | -1.3 | 114 |
| GlcN\text{Ac})-Lac-Ala-Glu-DAP | 692.315 | 692.310 | -0.6 | 860 |
| GlcN\text{Ac})-Lac-Ala-Glu-DAP | 668.298 | 668.299 | 1.6 | 6970 |
| GlcN\text{Ac})-Lac-Ala-Glu-DAP | 650.291 | 650.288 | -0.4 | 6495 |
| Lac-Ala-Glu-DAP | 635.289 | 635.289 | -0.5 | 324 |
| Glu-DAP | 621.278 | 621.273 | -0.7 | 1912 |
| Glu-DAP | 565.264 | 565.268 | 0.8 | 800 |
| Glu-DAP | 492.233 | 492.231 | -0.4 | 3051 |
| Glu-DAP | 478.208 | 478.204 | -0.9 | 449 |
| GlcN\text{Ac})-Lac-Ala-Glu-DAP | 463.205 | 463.204 | -2.7 | 521 |
| DAP-Glu-Ala | 391.185 | 391.183 | -0.5 | 608 |
| DAP-Glu | 373.173 | 373.172 | -0.8 | 1284 |
| DAP-Glu | 363.190 | 363.188 | -0.4 | 1374 |
| DAP-Glu | 349.162 | 349.161 | -0.1 | 2576 |
| DAP-Glu | 320.146 | 320.146 | -0.4 | 1973 |
| DAP-Glu | 302.136 | 302.135 | -1.6 | 3703 |
| DAP-Glu | 278.126 | 278.124 | -0.6 | 576 |
Supplementary Data 3.3: Tandem mass spectrometry analysis of the Tri→Tri dimer containing a uniformly labeled disaccharide-tripeptide cross-linked to a uniformly unlabeled disaccharide-tripeptide (3→3 cross-link). The observed \( m/z_{\text{obs}} \) and calculated \( m/z_{\text{calc}} \) values of the parental ion, \([M+2H]^2+\), as determined in the absence of fragmentation (MS1), are indicated. The \( m/z_{\text{calc}} \) value was used to select the ion for fragmentation. The difference between the \( m/z_{\text{obs}} \) and \( m/z_{\text{calc}} \) values is indicated in ppm. The GlcN residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcNRed), the acetyl group (Ac), and the D-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively.

The fragmentation potentially leads to two series of fragments (discriminatory fragments) specific of the isotopomers containing the fully labeled disaccharide subunit in the donor (all light-all heavy) or acceptor position (all light-all heavy). These fragments are highlighted by green and orange dots in the mass spectrum, respectively. The other fragments are common to the fragmentation of the two isotopomers (highlighted by blue dots in the mass spectrum). The mass spectrum indicates the presence of the all light-all heavy isotopomers (orange dots). The peak at \( m/z_{\text{obs}} \) 681.358 (green dot) can also be accounted for by the loss of \( \text{H}_2\text{O} \) from the peak at 699.367 indicating that all peaks may be accounted for by the presence of the all light-all heavy isotopomer.

In the mass spectrum, peaks differing by the loss of \( \text{H}_2\text{O} \) are connected by a dashed line. The arrows indicate the position of the 3→3 and 4→3 crosslinks. Residues in the acceptor are underlined.

An interactive report of the MS2 analysis is available in Supplementary File F3[3].
Supplementary Data 4.1: Tandem mass spectrometry analysis of the uniformly labeled Tetra(4→3)Tri dimer. The observed \( m/z_{obs} \) and calculated \( m/z_{calc} \) values of the parental ion, \([M+2H]^2+\), as determined in the absence of fragmentation (MS1), are indicated. The \( m/z_{calc} \) value was used to select the ion for fragmentation. The difference between the \( m/z_{obs} \) and \( m/z_{calc} \) values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcN\text{Red}), the acetyl group (Ac), and the D-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively. The peaks corresponding to the list of fragments appearing in the table are highlighted by orange dots in the mass spectrum.

In the mass spectrum, peaks differing by the loss of \( \text{H}_2\text{O} \) are connected by a dashed line. The tables only contain the \( m/z \) values for the fragments containing \( \text{H}_2\text{O} \). The arrows indicate the position of the 3→3 and 4→3 crosslinks. Residues in the acceptor are underlined.

An interactive report of the MS2 analysis is available in Supplementary File F4.1.

| Precursor ion (MS1)          | \( m/z_{obs} \) [M+2H]^2+ | \( m/z_{calc} \) [M+2H]^2+ | ppm | Intensity (a.u.) |
|------------------------------|-----------------------------|-----------------------------|-----|-----------------|
| GlcN(-Ac)-GlcN\text{Red}(-Ac)-Lac-Ala-Glu-DAP-Ala ➔ DAP-Glu-Ala-Lac-GlcN\text{Red}(-Ac)-GlcN(-Ac) | 939.992 | 939.992 | 0.1 | |

| Product ion                  | \( m/z_{obs} \) [M+H]^+ | \( m/z_{calc} \) [M+H]^+ | ppm | Intensity (a.u.) |
|------------------------------|--------------------------|--------------------------|-----|-----------------|
| GlcN(-Ac)-GlcN\text{Red}(-Ac)-Lac-Ala-Glu-DAP-Ala ➔ DAP-Glu-Ala-Lac-GlcN\text{Red}(-Ac) | 1666.880 | 1666.872 | -4.9 | 113 |
| GlcN\text{Red}(-Ac)-Lac-Ala-Glu-DAP-Ala ➔ DAP-Glu-Ala-Lac-GlcN\text{Red}(-Ac) | 1454.780 | 1454.769 | -7.6 | 462 |
| GlcN\text{Red}(-Ac)-Lac-Ala-Glu-DAP-Ala ➔ DAP-Glu | 1165.627 | 1165.619 | -6.9 | 103 |
| GlcN\text{Red}(-Ac)-Lac-Ala-Glu-DAP-Ala ➔ DAP-Glu | 1090.570 | 1090.574 | 3.6 | 240 |
| Ala-Glu-DAP-Ala ➔ DAP-Glu | 955.520 | 955.518 | -2.5 | 170 |
| Ala ➔ DAP-Glu-Ala-Lac-GlcN\text{Red}(-Ac) | 801.426 | 801.424 | -2.3 | 43 |
| GlcN\text{Red}(-Ac)-Lac-Ala-Glu-DAP-Ala ➔ Ala | 774.416 | 774.416 | -0.7 | 342 |
| GlcN\text{Red}(-Ac)-Lac-Ala-DAP-Ala ➔ Ala | 756.413 | 756.405 | -11.1 | 181 |
| Glu-DAP-Ala ➔ DAP-Glu | 726.390 | 726.380 | -13.5 | 50 |
| DAP-Glu-Ala-Lac-GlcN\text{Red}(-Ac) | 699.369 | 699.371 | 2.9 | 166 |
| GlcN\text{Red}(-Ac)-Lac-Ala-Glu-DAP | 681.366 | 681.361 | -6.8 | 54 |
| Ala-Glu-DAP | 392.216 | 392.211 | -12.7 | 44 |
| GlcN(-Ac) | 213.112 | 213.111 | -5.5 | 1238 |
Supplementary Data 4.2: Tandem mass spectrometry analysis of the uniformly unlabeled Tri(4→3)Tri dimer. The observed \( m/z_{\text{obs}} \) and calculated \( m/z_{\text{calc}} \) values of the parental ion, \([M+2H]^2+\), as determined in the absence of fragmentation (MS1), are indicated. The \( m/z_{\text{calc}} \) value was used to select the ion for fragmentation. The difference between the \( m/z_{\text{obs}} \) and \( m/z_{\text{calc}} \) values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcN\text{Red}), the acetyl group (Ac), and the D-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively. The peaks corresponding to the list of fragments appearing in the table are highlighted by orange dots in the mass spectrum.

In the mass spectrum, peaks differing by the loss of H\(_2\)O are connected by a dashed line. The tables only contain the \( m/z \) values for the fragments containing H\(_2\)O.

The arrows indicate the position of the 3→3 and 4→3 crosslinks. Residues in the acceptor are underlined.

An interactive report of the MS2 analysis is available in Supplementary File F4.2.

| Precursor ion (MS1) | \( m/z_{\text{obs}} \) [M+2H]^2+ | \( m/z_{\text{calc}} \) [M+2H]^2+ | ppm | Intensity (a.u.) |
|---------------------|-------------------|-------------------|-----|-----------------|
| GlcN(\text{Ac})-GlcN\text{Red}(\text{Ac})-Lac-Ala-Glu-DAP-Ala | 1551.693 | 1591.696 | 1.9 | 144 |
| GlcN\text{Red}(\text{Ac})-Lac-Ala-Glu-DAP-Ala | 1388.622 | 1388.617 | -0.3 | 1802 |
| GlcN\text{Red}(\text{Ac})-Lac-Ala-Glu-DAP-Ala | 1111.500 | 1111.501 | 0.4 | 395 |
| GlcN\text{Red}(\text{Ac})-Lac-Ala-Glu-DAP-Ala | 1040.474 | 1040.464 | -0.3 | 1464 |
| GlcN\text{Red}(\text{Ac})-Lac-Ala-Glu-DAP-Ala | 911.426 | 911.421 | -5.6 | 677 |
| Ala-Glu-DAP-Ala | 763.357 | 763.347 | -1.1 | 209 |
| Ala-Glu-DAP-Ala | 721.325 | 721.326 | 0.5 | 1560 |
| Glu-DAP-Ala | 692.308 | 692.310 | 3.2 | 291 |
| Glu-DAP-Ala | 668.297 | 668.299 | 2.4 | 618 |
| GlcN(\text{Ac})-Lac-Ala-Glu-DAP | 650.288 | 650.288 | 1.3 | 370 |
| Ala-Glu-DAP-Ala | 634.304 | 634.305 | 1.7 | 218 |
| Glu-DAP-Ala | 563.267 | 563.268 | 1.2 | 409 |
| Ala-Glu-DAP-Ala | 462.221 | 462.220 | -1.1 | 137 |
| Ala-Glu-DAP-Ala | 434.225 | 434.225 | -0.1 | 298 |
| Ala-Glu-DAP-Ala | 391.183 | 391.183 | 0.3 | 451 |
| Ala-Glu-DAP-Ala | 373.173 | 373.172 | -0.3 | 355 |
| GlcN\text{Red}(\text{Ac})-Lac-Ala | 349.161 | 349.161 | -1.1 | 557 |
| Glu-DAP-Ala | 320.146 | 320.146 | -1.6 | 252 |
| Glu-DAP-Ala | 302.135 | 302.135 | -0.7 | 366 |
| GlcN\text{Red}(\text{Ac})-Lac | 278.125 | 278.124 | -5.0 | 107 |
Supplementary Data 4.3: Tandem mass spectrometry analysis of the Tetra→Tri dimer containing a uniformly labeled disaccharide-tetrapeptide cross-linked to a uniformly unlabeled disaccharide-tripeptide (4→3 cross-link). The observed \( m/z_{\text{obs}} \) and calculated \( m/z_{\text{calc}} \) values of the parental ion, \([M+2H]^{2+}\), as determined in the absence of fragmentation (MS1), are indicated. The \( m/z_{\text{calc}} \) value was used to select the ion for fragmentation. The difference between the \( m/z_{\text{obs}} \) and \( m/z_{\text{calc}} \) values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcNRed), the acetyl group (Ac), and the \( \delta \)-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively.

In the mass spectrum, peaks differing by the loss of \( H_2O \) are connected by a dashed line. The arrows indicate the position of the 3→3 and 4→3 crosslinks. Residues in the acceptor are underlined.

An interactive report of the MS2 analysis is available in Supplementary File F4.3.
Supplementary Data 5.1: Tandem mass spectrometry analysis of the uniformly labeled Tri(4→3)Tetra dimer. The observed \( m/z \) \(_{\text{obs}} \) and calculated \( m/z \) \(_{\text{calc}} \) values of the parental ion, \([M+2H]^{2+}\), as determined in the absence of fragmentation (MS1), are indicated. The \( m/z \) \(_{\text{calc}} \) value was used to select the ion for fragmentation. The difference between the \( m/z \) \(_{\text{obs}} \) and \( m/z \) \(_{\text{calc}} \) values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcN\(^{\text{Red}}\)), the acetyl group (Ac), and the D-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively. The peaks corresponding to the list of fragments appearing in the table are highlighted by orange dots in the mass spectrum. In the mass spectrum, peaks differing by the loss of \( \text{H}_2\text{O} \) are connected by a dashed line. The tables only contain the \( m/z \) values for the fragments containing \( \text{H}_2\text{O} \). The arrows indicate the position of the 3→3 and 4→3 crosslinks. Residues in the acceptor are underlined. An interactive report of the MS2 analysis is available in Supplementary File F5.1.

| Precursor ion (MS1) | \( m/z \) \(_{\text{obs}} \) \([M+2H]^{2+}\) | \( m/z \) \(_{\text{calc}} \) \([M+2H]^{2+}\) | ppm | Intensity (a.u.) |
|---------------------|---------------------------------|---------------------------------|-----|-----------------|
| GlcN(−Ac)−GlcN\(^{\text{Red}}\)(−Ac)−Lac−Ala−Glu−DAP→DAP(−Ala)−Glu−Ala−GlcN\(^{\text{Red}}\)(−Ac)−GlcN(−Ac) | 939.988 | 939.992 | -3.3 | |
| Product ion | \( m/z \) \(_{\text{obs}} \) \([M+H]^+\) | \( m/z \) \(_{\text{calc}} \) \([M+H]^+\) | ppm | Intensity (a.u.) |
|---------------------|---------------------------------|---------------------------------|-----|-----------------|
| GlcN\(^{\text{Red}}\)(−Ac)−Lac−Ala−Glu−DAP→DAP(−Ala)−Glu−Ala−GlcN\(^{\text{Red}}\)(−Ac)−GlcN(−Ac) | 1454.757 | 1454.769 | 8.0 | 376 |
| GlcN\(^{\text{Red}}\)(−Ac)−Lac−Ala−Glu−DAP→DAP(−Ala)−Glu−Ala | 1165.632 | 1165.619 | -11.3 | 41 |
| GlcN\(^{\text{Red}}\)(−Ac)−Lac−Ala−Glu−DAP→DAP(−Ala)−Glu | 1090.586 | 1090.574 | -10.9 | 238 |
| GlcN\(^{\text{Red}}\)(−Ac)−Lac−Ala−Glu−DAP→DAP−Glu−Ala | 1072.561 | 1072.564 | 2.6 | 158 |
| GlcN\(^{\text{Red}}\)(−Ac)−Lac−Ala−Glu−DAP→DAP−Glu | 997.520 | 997.520 | 0.5 | 97 |
| GlcN\(^{\text{Red}}\)(−Ac)−Lac−Ala−Glu−DAP→DAP−(−Ac) | 955.528 | 955.518 | -10.1 | 135 |
| DAP(−Ala)−Glu−Ala−Lac−GlcN\(^{\text{Red}}\)(−Ac)−GlcN(−Ac) | 774.414 | 774.416 | 2.5 | 184 |
| Glu−DAP→DAP(−Ac) | 591.320 | 591.324 | 6.3 | 44 |
| GlcN\(^{\text{Red}}\)(−Ac)−Lac−Ala | 365.201 | 365.202 | 1.9 | 135 |
| Glu−DAP | 317.166 | 317.167 | 3.2 | 123 |
Supplementary Data 5.2: Tandem mass spectrometry analysis of the uniformly unlabeled Tri(3→3)Tetra dimer. The observed \( m/z_{\text{obs}} \) and calculated \( m/z_{\text{calc}} \) values of the parental ion, \([M+2H]^{2+}\), as determined in the absence of fragmentation (MS1), are indicated. The \( m/z_{\text{calc}} \) value was used to select the ion for fragmentation. The difference between the \( m/z_{\text{obs}} \) and \( m/z_{\text{calc}} \) values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcN\text{Red}), the acetyl group (Ac), and the D-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively. The peaks corresponding to the list of fragments appearing in the table are highlighted by orange dots in the mass spectrum. In the mass spectrum, peaks differing by the loss of H\text{2}O are connected by a dashed line. The tables only contain the \( m/z \) values for the fragments containing H\text{2}O. The arrows indicate the position of the 3→3 and 4→3 crosslinks. Residues in the acceptor are underlined.

An interactive report of the MS2 analysis is available in Supplementary File F5.[2]

| Precursor ion (MS1) | \( m/z_{\text{obs}} \) [M+H] | \( m/z_{\text{obs}} \) [M+2H] | Intensity (a.u.) |
|---------------------|---------------------------|---------------------------|-----------------|
| GlcN\text{(Ac)}-GlcN\text{Red}(Ac)-Lac-Ala-Glu-DAP > DAP\text{(Ala)}-Glu-Ala-Lac-GlcN\text{Red}(Ac)-GlcN\text{(Ac)} | 897.891 | 897.892 | -0.9 |
| GlcN\text{(Ac)}-Lac-Ala-Glu-DAP | 1591.703 | 1591.696 | 4.5 | 290 |
| GlcN\text{Red}(Ac)-Lac-Ala-Glu-DAP > DAP\text{(Ala)}-Glu-Ala-Lac-GlcN\text{Red}(Ac)-GlcN\text{(Ac)} | 1388.618 | 1388.617 | -0.8 | 2966 |
| GlcN\text{Red}(Ac)-Lac-Ala-Glu-DAP > DAP\text{(Ala)}-Glu-Ala-Lac-GlcN\text{Red}(Ac)-GlcN\text{(Ac)} | 1299.575 | 1299.569 | -4.2 | 311 |
| GlcN\text{Red}(Ac)-Lac-Ala-Glu-DAP | 1183.529 | 1183.522 | -5.7 | 139 |
| GlcN\text{Red}(Ac)-Lac-Ala-Glu-DAP | 1111.500 | 1111.501 | 0.4 | 614 |
| GlcN\text{Red}(Ac)-Lac-Ala-Glu-DAP | 1094.502 | 1094.474 | -25.7 | 132 |
| GlcN\text{Red}(Ac)-Lac-Ala-Glu-DAP | 1040.465 | 1040.464 | -1.8 | 1697 |
| GlcN\text{Red}(Ac)-Lac-Ala-Glu-DAP | 1022.450 | 1022.453 | 2.8 | 687 |
| GlcN\text{Red}(Ac)-Lac-Ala-Glu-DAP | 951.412 | 951.416 | 4.5 | 820 |
| GlcN\text{Red}(Ac)-Lac-Ala-Glu-DAP > DAP\text{(Ala)} | 911.422 | 911.421 | -0.9 | 805 |
| Lac-Ala-Glu-DAP > DAP\text{(Ala)}-Glu-Ala | 906.399 | 906.406 | 7.8 | 112 |
| GlcN\text{Red}(Ac)-Lac-Ala-Glu-DAP > DAP\text{(Ala)}-Glu-Ala | 822.372 | 822.373 | 1.4 | 141 |
| DAP\text{(Ala)}-Glu-Ala-Lac-GlcN\text{Red}(Ac) | 763.350 | 763.347 | -3.4 | 207 |
| Glu-DAP > DAP\text{(Ala)}-Glu | 739.335 | 739.336 | 0.9 | 1449 |
| Glu-DAP > DAP\text{(Ala)}-Glu | 692.308 | 692.310 | 3.2 | 351 |
| Glu-DAP > DAP\text{(Ala)} | 674.295 | 674.300 | 6.8 | 209 |
| GlcN\text{Red}(Ac)-Lac-Ala-Glu-DAP | 650.291 | 650.288 | -3.1 | 639 |
| Ala-Glu-DAP > DAP\text{(Ala)}-Glu | 634.304 | 634.305 | 1.8 | 231 |
| Glu-DAP > DAP\text{(Ala)}-Glu | 602.267 | 602.263 | -6.7 | 231 |
| Glu-DAP > DAP\text{(Ala)} | 563.270 | 563.268 | -4.0 | 605 |
| Ala-Glu-DAP > DAP\text{(Ala)} | 545.260 | 545.257 | -4.6 | 275 |
| GlcN\text{Red}(Ac)-Lac-Ala-Glu | 478.201 | 478.204 | 5.9 | 120 |
| Glu-DAP > DAP\text{(Ala)} | 474.220 | 474.220 | -1.0 | 198 |
| Glu-DAP > DAP\text{(Ala)}-Glu | 434.225 | 434.225 | -0.2 | 182 |
| DAP\text{(Ala)}-Glu | 391.180 | 391.183 | 7.9 | 360 |
| Ala-Glu-DAP | 373.173 | 373.172 | -2.4 | 360 |
| GlcN\text{Red}(Ac)-Lac-Ala | 349.160 | 349.161 | 3.2 | 653 |
| Glu-DAP | 302.135 | 302.135 | -0.7 | 1087 |
| GlcN\text{Red}(Ac)-Lac | 278.124 | 278.124 | 0.3 | 167 |
Supplementary Data 5.3: Tandem mass spectrometry analysis of the Tri→Tetra dimer containing a uniformly labeled disaccharide-tetrapeptide cross-linked to a uniformly unlabeled disaccharide-tripeptide (3→3 cross-link). The observed $m/z_{\text{obs}}$ and calculated $m/z_{\text{cal}}$ values of the parental ion, [M+2H]$^{2+}$, as determined in the absence of fragmentation (MS1), are indicated. The $m/z_{\text{cal}}$ value was used to select the ion for fragmentation. The difference between the $m/z_{\text{obs}}$ and $m/z_{\text{cal}}$ values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcN$^{\text{Red}}$), the acetyl group (Ac), and the d-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively.

In the mass spectrum, peaks differing by the loss of $\text{H}_2\text{O}$ are connected by a dashed line. The arrows indicate the position of the 3→3 and 4→3 crosslinks. Residues in the acceptor are underlined.

An interactive report of the MS2 analysis is available in Supplementary File F5.3.
Tetra-Tetra

Supplementary Data 6.1: Tandem mass spectrometry analysis of the uniformly labeled Tetra(4→3)Tetra dimer. The observed $m/z_{obs}$ and calculated $m/z_{calc}$ values of the parental ion, $[M+2H]^2+$, as determined in the absence of fragmentation (MS1), are indicated. The $m/z_{calc}$ value was used to select the ion for fragmentation. The difference between the $m/z_{obs}$ and $m/z_{calc}$ values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcN<sup>Red</sup>), the acetyl group (Ac), and the D-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively. The peaks corresponding to the list of fragments appearing in the table are highlighted by orange dots in the mass spectrum. In the mass spectrum, peaks differing by the loss of H<sub>2</sub>O are connected by a dashed line. The tables only contain the $m/z$ values for the fragments containing H<sub>2</sub>O. The arrows indicate the position of the 3→3 and 4→3 crosslinks. Residues in the acceptor are underlined. An interactive report of the MS2 analysis is available in Supplementary File F6.[I]
Supplementary Data 6.2: Tandem mass spectrometry analysis of the uniformly unlabeled Tetra(4→3)Tetra dimer. The observed \(m/z\) values and calculated \(m/z\) values of the parent ion, \([M+2H]^2+\), as determined in the absence of fragmentation (MS1), are indicated. The \(m/z\) value was used to select the ion for fragmentation. The difference between the \(m/z\) values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcN\text{Red}), the acetyl group (Ac), and the D-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively. The peaks corresponding to the list of fragments appearing in the table are highlighted by orange dots in the mass spectrum. In the mass spectrum, peaks differing by the loss of H\(_2\)O are connected by a dashed line. The tables only contain the \(m/z\) values for the fragments containing H\(_2\)O. The arrows indicate the position of the 3→3 and 4→3 crosslinks. Residues in the acceptor are underlined.

An interactive report of the MS2 analysis is available in Supplementary File F6[2].
Supplementary Data 6.3: Tandem mass spectrometry analysis of the Tetra→Tetra dimer containing a uniformly labeled disaccharide-tripeptide cross-linked to a uniformly unlabeled disaccharide-tripeptide (4→3 cross-link). The observed $m/z_{obs}$ and calculated $m/z_{calc}$ values of the parental ion, [M+2H]$^{2+}$, as determined in the absence of fragmentation (MS1), are indicated. The $m/z_{calc}$ value was used to select the ion for fragmentation. The difference between the $m/z_{obs}$ and $m/z_{calc}$ values is indicated in ppm. The GlcNAc residue is indicated in two moieties, glucosamine (GlcN) and the acetyl group (Ac), since these moieties are potentially differentially labeled. For the same reason, the reduced (Red) MurNAc residue is indicated in three moieties, reduced glucosamine (GlcN$^{\text{Red}}$), the acetyl group (Ac), and the D-lactoyl group. Labeled and unlabeled moieties are represented in red and purple, respectively. The fragmentation potentially leads to two series of fragments (discriminatory fragments) specific of the isotopomers containing the fully labeled disaccharide subunit in the donor (all heavy-all light) or acceptor position (all light-all heavy). These fragments are highlighted by green and orange dots in the mass spectrum, respectively. The other fragments are common to the fragmentation of the two isotopomers (highlighted by blue dots in the mass spectrum). The mass spectrum indicates the presence of the all light-all heavy isotopomers (orange dots). The peak at $m/z_{obs}$ 1128.568 (green dot) can also be accounted for by the loss of H$_2$O from the peak at 1142.567 indicating that all peaks may be accounted for by the presence of the all light-all heavy isotopomer.

In the mass spectrum, peaks differing by the loss of H$_2$O are connected by a dashed line. The arrows indicate the position of the 3→3 and 4→3 crosslinks. Residues in the acceptor are underlined.

An interactive report of the MS2 analysis is available in Supplementary File F6.3.
| Precursor ion (MS1) | m/z_{obs} [M+2H]^2+ | m/z_{calc} [M+2H]^2+ | ppm | Intensity (a.u.) | Isotopologue |
|---------------------|----------------------|----------------------|-----|-----------------|--------------|
| GlcN(-Ac)-GlcN\textsuperscript{26s}(-Ac)-Lac-Ala-Glu-DAP-Ala | 955.459 | 955.462 | -2.6 | all heavy—all light | |
| GlcN(-Ac)-GlcN\textsuperscript{26s}(-Ac)-Lac-Ala-Glu-DAP-Ala | 955.459 | 955.462 | -2.6 | all heavy—all light | |

| Discriminatory product ion | m/z_{obs} [M+H]^+ | m/z_{calc} [M+H]^+ | ppm | Intensity (a.u.) |
|----------------------------|-------------------|-------------------|-----|-----------------|
| GlcN\textsuperscript{26s}(-Ac)-Lac-Ala-Glu-DAP-Ala | 1128.568 | 1128.570 | 1.6 | 215 | all heavy—all light |
| GlcN\textsuperscript{26s}(-Ac)-Lac-Ala-Glu-DAP-Ala | 1053.516 | 1053.525 | 9.2 | 97 | all light—all heavy |
| Ala\textsuperscript{26s} | 845.449 | 845.453 | 4.3 | 304 | all light—all heavy |
| Ala\textsuperscript{26s} | 774.410 | 774.416 | 7.5 | 459 | all light—all heavy |
| Ala\textsuperscript{26s} | 752.394 | 752.398 | 5.1 | 307 | all light—all heavy |
| Ala\textsuperscript{26s} | 721.322 | 721.326 | 4.4 | 186 | all light—all heavy |
| GlcN\textsuperscript{26s}(-Ac)-Lac-Ala-Glu-DAP-Ala | 689.334 | 689.331 | 3.8 | 139 | all light—all heavy |
| GlcN\textsuperscript{26s}(-Ac)-Lac-Ala-Glu-DAP-Ala | 410.225 | 410.221 | -9.5 | 158 | all light—all heavy |
| GlcN\textsuperscript{26s}(-Ac)-Lac-Ala-Glu-DAP-Ala | 388.202 | 388.204 | 4.3 | 128 | all light—all heavy |
| GlcN\textsuperscript{26s}(-Ac)-Lac-Ala-Glu-DAP-Ala | 346.197 | 346.202 | 14.6 | 106 | all light—all heavy |

| Common product ion | m/z_{obs} [M+H]^1+ | m/z_{calc} [M+H]^1+ | ppm | Intensity (a.u.) |
|--------------------|-------------------|-------------------|-----|-----------------|
| GlcN\textsuperscript{26s}(-Ac)-Lac-Ala-Glu-DAP-Ala | 1494.729 | 1494.733 | 2.8 | 1109 |
| GlcN\textsuperscript{26s}(-Ac)-Lac-Ala-Glu-DAP-Ala | 1205.576 | 1205.583 | 6.2 | 113 |
| GlcN\textsuperscript{26s}(-Ac)-Lac-Ala-Glu-DAP-Ala | 1146.576 | 1146.580 | 3.8 | 295 |
| GlcN\textsuperscript{26s}(-Ac)-Lac-Ala-Glu-DAP-Ala | 1130.533 | 1130.539 | 5.7 | 219 |
| GlcN\textsuperscript{26s}(-Ac)-Lac-Ala-Glu-DAP-Ala | 1017.530 | 1017.538 | 7.3 | 126 |
| GlcN\textsuperscript{26s}(-Ac)-Lac-Ala-Glu-DAP-Ala | 995.475 | 995.483 | 7.4 | 176 |
| GlcN\textsuperscript{26s}(-Ac)-Lac-Ala-Glu-DAP-Ala | 647.322 | 647.329 | 11.3 | 149 |
| GlcN\textsuperscript{26s}(-Ac)-Lac-Ala-Glu-DAP-Ala | 302.130 | 302.135 | 15.7 | 19 | |
| GlcN(-Ac) | 204.089 | 204.087 | -9.0 | 2307 | |
| GlcN(-Ac) | 182.108 | 182.110 | 12.0 | 134 | |
