Proteins or peptides were an obvious choice to meet the need for calibrants during early mass spectrometry studies of high mass ranges (m/z > 1000) because these large biomolecules were widely available and monodisperse. However, they typically exhibited poor stability, and therefore required care during storage and sample preparation. While traditional linear polymers within a similar mass range can be easily prepared with enhanced stability, they exhibit polydispersity which can complicate accurate mass identification. Dendrimers, however, are a class of synthetic macromolecules that are prepared using an iterative step-wise synthesis, and therefore offer high molecular weights and monodispersity in addition to chemical robustness. A family of polyester dendrimers has been prepared and evaluated for use as calibrants.

Synthesis of Polymer Dendrimers

The dendrimers described in this study were synthesized using a divergent dendronization process initiated from one of four different core molecules (A, B, C, or D) and an acid anhydride monomer based on bis(hydroxy-methyl)propanoic acid or “bis-MPA” using previously reported techniques [1]. Each dendronization was continued through five synthetic iterations (or generations), yielding a total of 20 unique compounds.

Synthetic features

- Nearly quantitative reactions afford high purity.
- Simple purifications (extraction, precipitation, and filtration) enable large scale synthesis.
- Different cores can be used to access a range of MWs from 500 – 30,000 Da.

Representative molecular weights (theoretical values based on complete reactions)

<table>
<thead>
<tr>
<th>Generation</th>
<th>Molecular weight in Da C-28[Da(P-FS)m]n-28</th>
<th>Molecular weight in Da C-28[Di(Ph)n]n-28</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>1474,668</td>
<td>365,421</td>
</tr>
<tr>
<td>G2</td>
<td>3399,364</td>
<td>2342,989</td>
</tr>
<tr>
<td>G3</td>
<td>7240,876</td>
<td>5128,125</td>
</tr>
<tr>
<td>G4</td>
<td>14,923,90</td>
<td>10,058,450</td>
</tr>
<tr>
<td>G5</td>
<td>36,289,95</td>
<td>21,838,94</td>
</tr>
<tr>
<td>G6</td>
<td>61,022,04</td>
<td>44,120,03</td>
</tr>
<tr>
<td>G7</td>
<td>122,486,2</td>
<td>88,882,21</td>
</tr>
</tbody>
</table>

Why do dendrimers offer advantages as calibrants?

The highly branched structure imparts unique physical properties including enhanced solubility and reduced crystallinity which result in broad compatibility with matrix and solvent. Synthetic optimization yields truly monodisperse dendrimers with molecular weights up to 30,000 Da.

Dendritic Calibrant: Shelf-life Verification

The sterically hindered pivalate linkage in bis-MPA-based dendrimers is expected to impart chemical robustness and extended shelf-lives, relative to most synthetic polymers.

**“Dry Calibrant”**

G2 calibrant stored as powder

**“Dry Calibrant Mix”**

Mixture of G2 calibrant with 9-nitroanthracene matrix and Na+ counterion stored as powder

**“Wet Calibrant Mix”**

Mixture of G2 calibrant with 9-nitroanthracene and Na+ counterion stored in acetonitrile solution

The bis-MPA dendrimers exhibited multi-year shelf-lives, even when stored as mixture with matrix and counterion.

**Mass spectra of SpheriCal calibrants**

Figure 2. Representative structure of the SpheriCal calibrant with an average molecular weight [M + Na]+ of 15,171.2 Da. Mass spectra (below) obtained using a Bruker Ultraflex MALDI-TOF mass spectrometer.

Figure 3. Mass spectra for each of the five calibration sets providing a total of 20 evenly spaced calibration points from m/z 700–30,000. Observed ions are sodium adducts, [M+22.98]+.

Contact

Sigma-Aldrich Chemie GmbH
Dr. Norman Hardt
Industriestrasse 25
9471 Buchs SG, Switzerland
Phone: +41-81-7502206
Mail: norman.hardt@sial.com