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Macromolecular cages built by comparatively weak covalent bonds are demanding analytes for mass spectrometry, in particular when they have molecular masses in the 5–10 kDa range. The cages under study are either synthesized by linking the building blocks via boronic ester bond formation starting from boronic acids and phenols, or via imine bonds starting from amines and aldehydes. In some cases, reversible chain formation may occur causing a doubling of molecular weight [1,2].

Matrix-assisted laser desorption/ionization (MALDI) [3] still presents the most promising mass spectral technique for these giant cages as i) MALDI is sufficiently soft, ii) can ionize neutral species, and iii) MALDI spectra are governed by singly charged ions. Thus, MALDI spectra provide immediate overview of the synthetic progress, yield information on side products, and finally can prove the purity of a cage compound.

Synthesis of Macromolecular Cages

The cages were synthesized by connecting building blocks. The first pathway employs boronic ester bond formation starting from boronic acids and phenols, the second uses imine bonds starting from amines and aldehydes. Boronic ester cages are less stable as they are prone to hydrolysis. In some cases, boronic esters may undergo reversible chain opening and closing that can lead to catenane-type dimers [1,2].

Matrix Selection

Some cages were more tolerant to matrix selection than others. In case of the boronic ester cage below DCTB and dithranol worked almost equally well while CMBT just yielded a reasonable signal. Other cages only worked in DCTB and within a narrow range of matrix-to-analyte ratio. DCTB appears to work with all cages analyzed so far. DCTB is also compatible with SpheriCal dendrimers for mass calibration.

Experimental

- MALDI mass spectra were acquired using a Bruker Autoflex Speed LRF time-of-flight (TOF) mass spectrometer (Bruker Daltonik, Bremen, Germany).
- Generally, best spectra were obtained in positive–ion mode.
- Solutions of the macromolecular cages of 1–4 mg ml⁻¹ were admixed to solutions of the matrix at 10 mg ml⁻¹ in tetrahydrofuran or acetonitrile.
- As matrices, DCTB and occasionally dithranol served best. Sample and matrix solutions were mixed at a ratio of 1:5 to 1:40 and 1 µl of that solution was transferred onto a stainless steel sample holder and allowed to crystallize upon evaporation of the solvent.
- Some cages required careful optimization of the analyte–to–matrix ratio while others tolerated a wider range of mixing ratios.
- First, survey spectra were acquired in linear mode. Typically, methods to cover m/z 3000–15000 were used.
- Reflector mode spectra were acquired to obtain isotopically resolved signals, which could normally be achieved for cages up to 8–10 kDa.
- External mass calibration was either established on caesium iodide clusters [4] or on monodisperse SpheriCal dendrimers [5–6].

Linear versus Reflector

Both spectra show [M+H]⁺ and [M+Na]⁺ ion peaks of the cage. Only reflector mode delivers isotopic resolution. The calculated isotopic pattern of C₅₀H₆₅N₃S₃ corresponds to the [M+H]⁺ ion signal. The most intensive peak of the pattern appears at m/z 5286.589.

Tracking Reactions

Building complex cages does not necessarily proceed as smoothly as intended. MALDI–TOF spectra provide valuable information even in case of only partial reaction or formation of complex mixtures, e.g., partial condensation can be recognized and tracked. Thus, MALDI–TOF–MS provides a quick and facile tool for optimization of reaction conditions to support syntheses of cage compounds.
Good shot-to-shot reproducibility of this preparation allowed the instrument to be optimized for resolving power at given m/z. While isotopic resolution could well be achieved across the m/z 250–7500 range, signals of SpheriCal-4C were at the limit of mass resolving power and those of SpheriCal-4D were just beyond. Nonetheless, suitable settings of the delay allowed to shift the m/z of optimum resolution to either m/z 9940 or m/z 12400. A delay of 220 ns presented the best compromise for the higher m/z range.

### Macromolecular cages of either boronic ester or imine–linked type can be analyzed by MALDI–TOF–MS preferably in positive-ion mode using DCTB matrix.

- Positive-ion mode normally suited best.
- Solutions of the cages at 1–4 mg ml$^{-1}$ were admixed to DCTB or dithranol matrix solutions (10 mg ml$^{-1}$) and spotted onto stainless steel sample targets.
- The ratio of analyte to matrix solution varied from 1 : 5 to 1 : 50. Some samples required careful optimization of the ratio, others were tolerant.

### Positive-ion MALDI–TOF reflector mode spectrum of SpheriCal 3 (PFS-13) and SpheriCal 4 (PFS-14). Each dendrimer forms [M+Na]$^+$ and [M+Cs]$^+$ ions leading to eight reference peaks in total. Inserts show expanded views of the isotopic patterns of [M+Cs]$^+$ ion signals. Enhanced cubic calibration based on the reference peaks listed above (top right) yielded a standard deviation of the calibration curve of 4.7 ppm.

### References


### Take-Home Message

- About 0.1 u mass accuracy can be achieved employing SpheriCal monodisperse dendrimer standards for external mass calibration.

### Procedure and Instrument Settings

- All MALDI–TOF spectra were acquired using a Bruker Autoflex Speed LRF instrument (optimized settings below).
- Solutions of the cages at 1–4 mg ml$^{-1}$ were admixed to DCTB or dithranol matrix solutions (10 mg ml$^{-1}$) and spotted onto stainless steel sample targets.
- The ratio of analyte to matrix solution varied from 1 : 5 to 1 : 50. Some samples required careful optimization of the ratio, others were tolerant.
- Survey spectra were first acquired in linear mode.
- Well-defined cages were then analyzed in reflector mode.
- Spectra of cages up to 8–10 kDa showed isotopic resolution in reflector mode.
- SpheriCal standards were advantageous for instrument tuning and mass calibration.
- Calibration in linear mode was based on relative molecular masses of SpheriCal standards while reflector mode calibration was based on individual isotopic peaks.
- Reference mass lists and method files (for Bruker FlexControl, optimized for Autoflex) are available from the presenting author upon request.

### Limits of Resolving Power

- Positive-ion MALDI–TOF reflector mode spectrum of SpheriCal 3 (PFS-13). Each dendrimer forms [M+Na]$^+$ and [M+Cs]$^+$ ions leading to eight reference peaks in total. Inserts show expanded views of the isotopic patterns of [M+Cs]$^+$ ion signals. Enhanced cubic calibration based on the reference peaks listed above (top right) yielded a standard deviation of the calibration curve of 4.7 ppm.

### General Characteristics

- MALDI–TOF–MS yielded useful mass spectra of the cages under study. The compounds were typically found to either form molecular ions, [M]$^+$, or protonated molecules, [M+H]$^+$. Cationization with alkali metal ions or ammonium occurred less frequently.
- Boron ester cages often comprising 24 borons resulted in complex isotopic distributions. Achieving sufficient resolution turned out as a key requirement for this class of cages. Optimization of the ion source lens voltage and the delay time of the pulsed ion extraction allowed a substantial increase of the resolving power.
- The use of monodisperse dendrimers [5] provided valuable aid in tuning and mass calibration of the instrument [6].

### Achieving Isotopic Resolution

- Semming small changes in molecular structure exerted marked influence on the intensity of the molecular ion peak and on the occurrence of fragment ion peaks. While some compounds were stable enough to form ions without concomitant fragmentation others exhibited intensive fragment ion peaks.
- Particularly, the introduction of alkyl substituent at the bridging position of trypticene building blocks resulted in multiple losses of alkene molecules.

- Of optimum resolution to either m/z 9940 or m/z 12400. A delay of 220 ns presented the best compromise for the higher m/z range.

### Take-Home Message

- About 0.1 u mass accuracy can be achieved employing SpheriCal monodisperse dendrimer standards for external mass calibration.

### Procedure and Instrument Settings

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