A Novel MALDI-MS Approach for the Analysis of Neutral Metallosupramolecular Architectures


Keywords: Supramolecular chemistry / Mass spectrometry / Noncovalent interactions / Metallogrids / Analytical methods

Matrix assisted laser desorption/ionisation mass spectrometry (MALDI-MS) methods have been developed for the characterisation of neutral \([2 \times 2]\) metallogrids derived from diimine, dihydrazone and diacylhydrazone ligands. Such grids may be protonated in solution to give cationic species but in most cases these are labile, so that rather delicate conditions are required for observation of the intact metallogrids as monoprotonated derivatives in the gas phase. As a MALDI matrix, 2,4,6-trihydroxyacetophenone (THAP) is sufficiently acidic to enable monoprotonation of the grids unaccompanied by dissociation, and if the grid sample is initially deposited by a layering technique to avoid any preliminary dissociation in solution the mass spectrum of the intact mono-protonated grid is readily obtainable. The stoichiometry of 24 grids obtained from several different ligands and metals was confirmed with this optimized protocol. The deposition technique used means that the best signal-to-noise ratio in the spectra is obtained with only a small number of laser shots (ca. 5) to volatilise the sample. This MALDI-MS protocol can be applied to the study of grid dissociation in solution and in the case of CuII grids, in particular, has revealed the formation of various unusual clusters. The crystal structure is reported of one such cluster that was isolated and contains a nonionisable ligand that is related to that incorporated within the grids analysed by the MALDI-MS protocol.

Introduction

Metallosupramolecular chemistry has generated a variety of inorganic architectures by self-assembly processes with suitably designed ligands and specific metal ions.[1–8] Amongst the remarkably diverse range of structures now known, an important group is that of the (metallo)grids in which an essentially planar regular array of metal ions is constrained by rigid polytopic ligands.[9–15] The most abundant of these grids are those of the \([2 \times 2]\) type formed by relatively readily synthesised ditopic ligands, and the present work is concerned with such grids, formed with the ligands \(L_1H_2–L_10H_2\) (Scheme 1), in their deprotonated forms i.e. as neutral \([M_4(L^n)]_4\) units, where M is a dipoisitive metal ion.

In regard to the possible application of these grids as receptors is solution, it is essential to know whether the grid structure, usually readily established in the solid state by single crystal X-ray diffraction measurements,[9–11,15] is indeed retained in solution and under what range of conditions this may be true. In some instances with diamagnetic grids, their 1D NMR spectra show characteristic splittings uniquely compatible with the grid structure,[9] but in other instances the apparent symmetry is higher than that expected for the grid, while in the spectra of most paramagnetic complexes peak broadening and the loss of coupling mean that the spectra cannot be used to establish unambiguously the grid form.[16] Diffusion ordered spectroscopy

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Scheme 1. Ligands that give rise to the grid complexes studied in the present work.
(DOSY) measurements may prove to be suitable for this but there is uncertainty in relating diffusion coefficients to the nuclearity of nonspherical species, which is probably the state of the grid complexes in solution.\(^{12,17}\) In principle, equilibrium measurements could be used to determine stability constants from which species distributions could be calculated, but the systems are extremely complicated to resolve and in practice it is nigh impossible to find a solvent suitable for all reaction components and thus enable measurements of homogeneous media. Hence, with advantages such as an insensitivity to the magnetic state of the complexes, the present study was initiated in the hope that mass spectrometry would provide a means to surmount most of these problems, at least to the extent of defining conditions under which the solid state species are retained in solution.

Over the past several years, electrospray ionization mass spectrometry (ESI-MS) has emerged as a powerful tool for the investigation of noncovalent biological complexes\(^{18–21}\) as well as supramolecular assemblies\(^{22–25}\) including grid-type complexes.\(^{26}\) However, ESI-MS only detects the charged species present in any solution and thus is unsuited to the characterisation of neutral species unless they are convertible to cations or anions without any other change in their composition. While certain grids do undergo reversible protonation/deprotonation reactions in solution,\(^ {26}\) grids derived from diacylhydrazones (Scheme 1, ligands \(L^3–L^4\)) are stable when the ligand is in its dianionic form but tend to dissociate when acid, sufficient to protonate the ligand, is present.\(^ {16}\) That MALDI-MS can provide a solution to the dilemma of how to obtain the mass spectrum of an intact neutral grid derivative was unanticipated, but in fact it appears that reaction between the neutral grids and the weakly acidic matrix materials can lead to the transfer of a single proton to each complete grid and thus to the generation of monocations that are sufficiently stable to be characterised by mass spectrometry. It is the fact that the proton transfer most certainly occurs in the MALDI-plume following laser excitation and not in solution that is the origin of the success of this method, since reaction between the matrix material and the grids in solution leads to extensive decomposition.\(^ {12,27}\) In contrast to some other metal complex systems,\(^ {28}\) here it appears that the special reactivity of a MALDI system can be advantageous.

It may be noted that MALDI-MS operating with adapted sample preparation and analysis conditions has been shown, since its development in 1988,\(^ {29}\) to be a very powerful technique for the analysis of compounds involved in noncovalent interactions, such as is commonly the case for proteins. It has been successfully used to characterise numerous multinuclear metal complexes,\(^ {30,31,32}\) and thus the present work defines an extension of its applicability.

Results and Discussion

Optimization of the MALDI Protocol

**MALDI Matrix Selection**

Three common MALDI matrices were tested in this study: \(\alpha\)-cyano-4-hydroxy-cinnamic acid (CHCA), 1,8,9-anthracencetiol (dithranol) and 2,4,6-trihydroxyacetophenone (THAP). THAP and dithranol are often used as matrices for acid-sensitive species because of their considerably lower acidities compared to other commonly used matrices such as CHCA. For the matrix selection, two grids derived from the acylhydrazone ligands \(L^4H^2_2\) and \(L^6H^2_2\), that of the former having been characterised crystallographically as a grid,\(^ {16}\) were chosen to provide a sensitive test of the method, as it is known that grids derived from the fully protonated (or \(N\)-alkylated) form of the dihydrazone ligand \(L^4H^2_1\) are sufficiently stable to be characterised by conventional ESI-MS methods (see ref.\(^ {34}\) and references cited therein). These initial experiments were conducted with the “layered sample” method (see below).

With THAP as the matrix, the mass spectrum of \([\text{Zn}_{4–}(L^6)_3]\) showed a single peak for a mass corresponding to that of the intact protonated grid \([L^6H^2_2Zn^2+H^+]\) \((m/z = 2224.8)\) (Figure 1). With either dithranol or CHCA as the matrix additional peaks were observed in the spectra of \([\text{Zn}_{4–}(L^4)_3]\). In the spectrum recorded with dithranol as the matrix, the peak for the free protonated ligand \([L^4H^2_2Zn^2+H^+]\) \((m/z = 493.2)\) was the most intense, indicating that dissociation of the complex had occurred while it was in the initial solution. In the spectrum measured with CHCA as the matrix, the free protonated ligand \([L^6H^2_2Zn^2+H^+]\) \((m/z = 493.3)\) provided the base peak, with additional peaks appearing for \([L^6Zn^2+H^+]\) (39\% ligand peak intensity) and for \([L^4Zn^2+H^+]\) (8\% ligand peak intensity). This indicates
that at least partial dissociation of the grid had occurred, with the peak for the intact protonated grid being extremely weak (7% of the peak intensity of the protonated ligand). Similar results were obtained for [Zn₄(L⁶)₄] with the same matrices (data not shown).

In all spectra recorded for these two grids and with all three matrices, peaks corresponding to the monoprotonated intact grids were detectable. However, with THAP as the matrix, fragmentation was minimised, and according to the currently accepted classification based on proton affinity, THAP is considered as a “colder” matrix than CHCA and dithranol (proton affinity: 201.0 kcal/mol for CHCA, 211.5 kcal/mol for dithranol, 213.3 kcal/mol for THAP[34]). Qualitative experiments with peptides indicate that a “hot” matrix leads to abundant analyte fragmentation whereas a “cold” matrix results in fewer or no fragment ions.[35,36] The same result is observed in the case of the grids under study. Thus, THAP was the preferred matrix for all subsequent experimentation.

**MALDI Sample Deposition Optimization**

Two sample deposition techniques were assessed with the grid [Zn₄(L⁶)₄] and THAP as the matrix. In the “layered sample” preparation technique, 1 µL of matrix solution was applied to the stainless steel plate, dried in air, and then covered by a second layer comprising 1 µL of analyte solution, which was allowed to dry in air. In the case of the “dried droplet” method, the sample was directly mixed in a 1:1 ratio (by volume) with the matrix solution, and then 1 µL aliquot of this solution was dried on the plate.

With the layered sample preparation technique, the mass spectrum showed a single peak corresponding to the intact protonated grid [Zn₄(L⁶)₄]+ (m/z = 2224.8) (Figure 2, a). With the dried droplet method (Figure 2, b), a peak for the protonated free ligand [L⁶H₂Zn⁺]+ (m/z = 493.2) appeared in the spectrum corresponding to 36% of the intact grid peak intensity. In the case of the layered sample preparation technique, the contact time for the two solutions was essentially zero, whereas the contact time was at least several minutes in the case of the dried droplet method. Further experiments were therefore conducted to assess the hypothesis that the contact time determined the extent of grid dissociation, viz. that there was relatively slow acid-catalysed decomposition of the grid induced by the matrix molecules in homogeneous solution.

Thus, with the dried droplet method the solution containing both grid and matrix was allowed to stand for 5, 20 and 60 min before aliquots were withdrawn and applied to the plate. The aliquots were allowed to dry rapidly in air before the mass spectra were recorded. In the spectrum recorded after the 5 min reaction period a weak but obvious peak for the protonated free ligand was detectable along with a peak for the monoprotonated grid (Figure 2, b), indicating that significant dissociation had occurred in this time. In the spectra recorded after reaction periods of 20 or 60 min the protonated ligand peaks had become the base peaks of the spectra (Figure 2, c and d), with peaks for [L⁶H₂Zn⁺]+ as well as for [Zn₄(L⁶)₄]+ being apparent in both, consistent with a state of partial dissociation equilibrium having been reached in both cases.

Given that these observations confirmed the important influence of reaction between the matrix and the grids in solution, and given also that the layered sample technique must minimise any such reaction (it cannot be excluded that some dissolution of the pre-dried matrix in the grid solution could occur during the period required for evaporation), all subsequent measurements employed the layered sample method.

**MALDI Laser Shot Optimization**

For biological complexes it has been shown that some clusters formed by noncovalent interactions can be observed by MALDI-MS but only when spectra are obtained from the first or the first few laser shots directed at nonirradiated sample positions, as the signals of the dissociated species dominate the spectra arising from subsequent irradiations.[37] This has been described as the “first-shot phenomenon”. [38] In this study, we investigated whether this first-shot phenomenon may also be characteristic of supramolecular chemical assemblies.

Figure 3 shows the spectra of the supramolecular grid [L⁶]₄Co₄ that were obtained with the layered sample preparation method and with THAP as the matrix (see Exp. Section). A total of 400 shots were accumulated for each spectrum. To preserve the intact structure of the grid, all spectra were recorded at minimal laser intensity, which is to say slightly above the threshold for desorption. We have
found that with high laser intensities cleavage of what is presumably the weaker bonds in the ligand, the N–N bonds, may occur.

MALDI-MS Analysis of Neutral Grids

Study of the Metal Ion Dependence of the Solution Speciation of the Grids

The optimised procedure for obtaining MALDI mass spectra described above was applied to some 24 neutral grids derived from the ligands shown in Scheme 1 and various transition metal ions. The results obtained are summarised in Table 1. While in all cases a peak for a tetrancular species corresponding to the expected grid was obtained, and in most instances this was the only peak in the spectrum, the spectrum of the CuII grid, \([L^6]_4\text{Cu}_4\), provided an exception. Note that this complex, along with its CoII, NiII and ZnII analogues, has been shown by X-ray crystallography to have a grid form in the solid state.\(^{[16]}\) Thus, the complexes \([L^6]_4\text{Ni}_4\) and \([L^6]_4\text{Zn}_4\) gave MALDI spectra in which there were essentially single peaks \([([L^6]_4\text{Ni}_4]^+ + \text{H}^+)(mlz = 2198.9)\) and \([([L^6]_4\text{Zn}_4]^+ + \text{H}^+)(mlz = 2224.8)\), respectively, corresponding to the monoprotonated tetrancular species (presumed to be the grids). The CuII complex, however, shows a far more complicated spectrum (Figure 4) with peaks corresponding to the species

Figure 3 (a) shows the spectrum obtained when only the first laser shot onto a new sample position is considered. In order to be able to change the laser shot position between two individual spectra, the laser pulse frequency was markedly reduced from 50 to 1 Hz. The accumulation of the 400 single spectra was performed with the instrument operating in an automatic mode so that each spectrum arose from a nonirradiated surface. Figure 3 (b to e) show the spectra obtained from the accumulation of data from the 2nd to the 11th shots onto a given position (3b), the 11th to the 20th (3c), the 21st to the 100th (3d), and the 101st to the 201st shots onto a given position; (e) sum of data from the 21st to the 101st shots onto a given position. (f) shows the spectrum obtained from the accumulation of data from the 2nd to the 11th shots onto a given position; (g) sum of data from the 11th to the 21st shots onto a given position; (h) sum of data from the 21st to the 101st shots onto a given position; (e) sum of data from the 101st to the 201st shots onto a given position.

Table 1. Summary of MALDI-MS results obtained for the different grids analysed.

<table>
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<tr>
<th>Grid</th>
<th>Formula</th>
<th>Calcd. isotopic masses(^{[a]}) [M + H(^+)] ([\text{Da}])</th>
<th>Measured masses [M + H(^+)] ([\text{Da}])</th>
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<td>([L^6]_4\text{Ni}_4)</td>
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<td>1801.32</td>
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<td>([L^6]_4\text{Mn}_4)</td>
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<td>2633.52</td>
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<td>2017.26</td>
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<td>2113.41</td>
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<td>6472.09(^{[b]})</td>
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<td>(\text{C}<em>{292}\text{H}</em>{256}\text{O}_{24}\text{Cu}_4)</td>
<td>3378.19(^{[b]})</td>
<td>3378.82(^{[b]})</td>
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\(^{[a]}\) Peak assignments were verified by comparing the experimental isotopic patterns to theoretical isotopic patterns. \(^{[b]}\) In this case, the calculated and measured masses are average masses (the observed mass of the grid is taken as the maximum of the signal formed by the overlap of the unresolved natural isotope peaks).
MALDI-MS Analysis of Metallosupramolecular Architectures

Figure 4. MALDI-TOF mass spectra of [(L₆)₄Zn₄⁺H⁺], [(L₆)₄Ni₄⁺H⁺] and [(L₆)₄Cu₄⁺H⁺], prepared with the layered sample preparation technique, measured in the positive ion mode with THAP as the matrix. Each spectrum is the sum of 500 shots. The y-scale is normalized to the most intense peak in each mass spectrum. (a) Metal: zinc; (b) metal: nickel; (c) metal: copper. In all cases the ligand/metal ratio equals 1.

[(L₆)₄Cu₄⁺H⁺], [(L₆)₃Cu₅⁺H⁺], [(L₆)₃Cu₄⁺H⁺⁺], [(L₆)₂Cu₄⁺H⁺⁺], [(L₆)₂Cu₃⁺H⁺⁺], [(L₆)₂Cu₂⁺H⁺⁺], [(L₆)²Cu⁺H⁺⁺] and [(L₆)²Cu⁺H⁺⁺] being present. This is consistent with the observation of marked distortions in the solid state structure of the Cu complex when compared to its transition metal analogues,[16] and is probably a reflection of the fact that a regular octahedral coordination geometry is essentially never observed for CuII. It is also possibly a reflection of the sensitivity of the CuII complex to acid, since when attempting to form the Cu(CF₃SO₃)₂ complex of the grid forming ligand L₁₂H₂ by condensing imidazole-2-aldehyde with 4,6-bis(N-methylhydrazino)-2-phenylpyrimidine the species actually isolated[39] was the complex [Cu₂(L₁₂)(L₁₂H)₂](CF₃SO₃)₆. The structure of the cation present in the crystal lattice of this complex[40] is shown in Scheme 2 (the structure of L₁₂ is also shown).

Note that the grids derived from the dihydrazone ligand L¹ and the diimine ligand L² are known[27,33] to be less sensitive to acid induced dissociation in solution than the grids derived from acylhydrazones,[16] and thus it is unsurprising that peaks for monoprotonated tetranuclear species were readily observed in their MALDI mass spectra. Nonetheless, the technique remains particularly useful for their characterisation. An interesting case in the acylhydrazone series is provided by the grid [(L⁹)₄Zn₄⁺], L⁹ being a derivative of L-alanine. Although efforts to obtain a crystal structure of this complex were unsuccessful, its ᵁH NMR spectrum, recorded with the complex in water, is an example of one that clearly supports the assignment of a grid structure to such a complex. The twofold rotational symmetry of the free ligand should be lost when it is incorporated in a grid and, consistent with this, the spectrum (Figure 5) shows two doublets for the alanine methyl groups and two hydrazone CH singlets. Hence, the NMR and mass spectra for this complex are complementary.
Figure 5. Structure of the ligand L9 and the NMR spectrum of [(L9)4Zn4].

Study of the Relative Stability of the Grids

While the use of 10^-3 m grid solutions prepared with 1:5 methanol:dichloromethane as the solvent enabled it to be shown in all cases, other than that for the CuII grids, that essentially only the intact tetranuclear grid species was present, use of more dilute solutions provided evidence that all the grids underwent dissociation in solution. Thus, when obtained under the same conditions used for collection of the data given in Figure 4, the spectrum of [(L6)4Zn4], for example, changed from a single peak for [(L6)4Zn4+H]+ (m/z = 2224.8) that is seen when the sample solution has an initial concentration of 10^-3 m to one showing a peak for the free protonated ligand [(L6H2)+H]+ (m/z = 493.2) that increases in intensity as the concentration of the solution was adjusted to 10^-4 m and below (Figure 6). By determining the concentration at which the free ligand peak could be detected in the spectra, a rough order of stability was established for a selection of six grids (Table 2) involving different ligands and different metals. The order of relative stability found was: [(L10)4Zn4] > [(L9)4Co4] > [(L7)4Co4] = [(L7)4Ni4] = [(L7)4Cu4] = [(L7)4Zn4], indicating that when in a relatively apolar solvent the grid stability may depend more on the nature of the ligand than that of the metal.

Table 2. Grid resistance to dissociation.a

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Metal ion</th>
<th>Dissociation concentration [m]</th>
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<td>[L4]2-</td>
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</tr>
<tr>
<td>[L6]2-</td>
<td>Co2+</td>
<td>10^-3</td>
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<tr>
<td>[L6]2-</td>
<td>Cu2+</td>
<td>10^-3</td>
</tr>
<tr>
<td>[L6]2-</td>
<td>Ni2+</td>
<td>10^-3</td>
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<tr>
<td>[L6]2-</td>
<td>Zn2+</td>
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</tr>
<tr>
<td>[L10]2-</td>
<td>Zn2+</td>
<td>10^-5</td>
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</table>

(a) The given concentrations correspond to those at which the free ligand peak can be detected in the mass spectrum.

Conclusions

In this study, we have successfully characterised neutral [2×2] grid complexes by MALDI-MS. This required the development of a procedure in which a matrix acidic enough to transfer a single proton to each of the grids but not so acidic as to lead to grid decomposition was used. A suitable matrix, at least for the large family of grids derived from hydrazone and acylhydrazone ligands, was found to be trihydroxyacetophenone (THAP). Its successful application, however, required the use of a sequential layering technique for the sample preparation so that any contact between the substrate and matrix was limited to that which occurs in the gas phase. The fact that the substrate is deposited in a very thin layer means that there is a rapid diminution of signal if the laser irradiation is concentrated on a single site.

The measurements that were possible as a result of the optimisation of this MALDI-MS technique have provided extremely valuable confirmation that the grid structure established from solid state studies is retained in solution, though they have also shown that grids derived from acylhydrazone ligands do undergo dissociation in dilute solutions (<10^-4 m). It should indeed be possible to exploit this new technique to unravel the details of grid formation and dissociation in solution, and to study in a broad sense the many factors that influence whether or not multitopic ligands form grid species in solution.[11b]

We have demonstrated for the first time the feasibility of characterising neutral supramolecular inorganic architectures by MALDI-MS under adapted sample preparation and analysis conditions. However, this is far from a routine matter: the optimal choice of matrix, preparation procedure, and instrumental parameters strongly depends on the noncovalent assembly of interest and involves optimization of these parameters for each type of noncovalent complex. A general procedure for the detection of such assemblies can therefore not yet be given.
Experimental Section

Instrumentation: MALDI-MS mass spectra were acquired on a time-of-flight mass spectrometer (MALDI-TOF-TOF Autoflex II TOF-TOF, Bruker Daltonics, Bremen, Germany) equipped with a nitrogen laser (λ = 337 nm) operating at a pulse rate of 1 Hz. Full scans were acquired under the following experimental conditions: pulsed ion extraction, 90 ms; reflector, 20 kV. All the spectra shown herein were obtained in reflection mode and positive ion mode. Scanning was performed over a m/z range of 280–3520; the low mass cut off was set in all cases to m/z = 280. Mass spectra were obtained by averaging over a large area, with the laser beam being scanned continuously and randomly over a spot on the sample. A total of 500 shots were accumulated for each spectrum, with a maximum of 5 shots on any one position.

The spectrometer was carefully tuned in order to preserve the non-covalent interactions within the samples and to make sure that the species detected faithfully reflected those present in solution. In particular, the laser irradiance was carefully chosen to avoid fragmentation. An increase in the laser irradiance would lead to fragmentation of any noncovalent bonds. The laser irradiance was generally slightly above the threshold for desorption.

An external multi-point calibration covering a m/z 400–3000 mass range was carried out before each measurement with the singly charged peaks of a standard peptide mixture (0.4 μm, in water acidified with 1% HCOOH).

Mass assignments were performed with unprocessed spectra to obtain optimal correlation between the observed and calculated masses. As a further check of the correctness of the mass assignments, signal attributions were verified by comparing the experimental isotopic profiles to isotopic simulations.

Scan accumulation and data processing were performed with the FlexAnalysis 3.0 software.

Materials: α-Cyano-4-hydroxycinnamic acid (CHCA) was obtained from Sigma (St Louis, MO, USA), 1,8-antihenecetrol (dithranol) from Alfa Aesar (Karlsruhe, Germany) and 2,4,6-trihydroxyacetophenone (THAP) from Fluka (Buchs, Switzerland).

Sample Preparation: Matrix solutions were freshly prepared before use to minimise chemical degradation, which reduces their effectiveness in the ionization process. CHCA and dithranol were dissolved in acetone to the point of saturation. THAP was used at a concentration of 0.1 M.

Samples for MALDI-MS were prepared by dissolving the complex under study in an appropriate solvent (commonly a dichloromethane/methanol mixture) at a concentration of 10⁻³ M.

Two alternative sample preparation techniques were also used:

1. The Layered Sample Preparation Technique: The matrix solution (1 μL) was applied to the stainless steel plate (Bruker Daltonics, Bremen, Germany), and the rapid evaporation of the solvent resulted in a homogeneous surface comprising very small crystals. A second layer composed of the analyte solution (1 μL) was then deposited onto this matrix surface and dried in air.

2. The Dried Droplet Method: The sample was mixed in a 1:1 ratio (by volume) with the matrix solution. This solution (1 μL) was then deposited and dried on the stainless steel plate.

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[40] $\left[\text{Cu}_2\left(\text{L}^{12}\right)_2\right]\left(\text{CF}_3\text{SO}_3\right)_{6}$: $C_{66}H_{62}Cu_{16}N_{14}O_{48}S_6$, $M_r 2224.9$ g/mol; triclinic, space group $P1$; $a = 18.782(4)$ Å, $b = 19.001(4)$ Å, $c = 20.477(4)$ Å, $α = 107.98(3)^\circ$, $β = 106.08(3)^\circ$, $γ = 108.05(3)^\circ$; $T = 123.0(1)$ K. For 12638 observed $I > 2σ(I)$ reflections, $R_1 = 0.129$, $wR_2 = 0.277$, $S = 1.057$. CCDC-7785083 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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