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# Easy protocol for making a good *E*-sinapinic acid matrix for neutral and sulfated carbohydrate MALDI-MS analysis†

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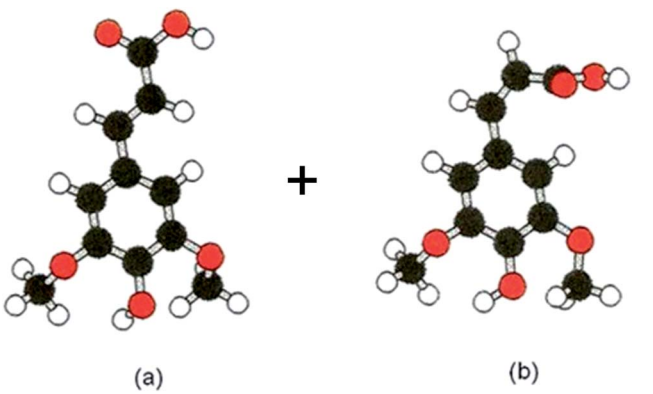
Since the introduction of 3,5-dimethoxy-4-hydroxycinnamic acid (SA) and α-cyano-4-hydroxycinnamic acid as matrices, the successful application of matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) started. Cinnamics can exist as two different geometric isomers, the *E*- and *Z*-forms. The commercially available cinnamics currently used as matrices are *E*-cinnamics; they do not perform well in general for carbohydrate analysis. Recently, *Z*-cinnamic acid properties for matrices were studied and compared with those of the corresponding *E*-isomer. For the analysis of neutral/sulfated carbohydrates the outstanding performance for *Z*-SA was demonstrated. As the synthesis of pure *Z*-cinnamic acids requires several steps and the manipulation of some not friendly chemicals (*i.e.*, bad smelling organic amines, toxic compounds, organic solvents, *etc.*), here we describe a convenient new one-pot protocol to prepare *in situ*, in a methanolic solution of commercial *E*-acid (*i.e.*, *E*-SA), a *Z*- + *E*- mixture by photoisomerization (UVB irradiation); then, the only step required is the addition of water to the irradiated solution to become ready as a matrix stock solution for MALDI experiments. This "photo-made at home" matrix performs carbohydrate analysis with similar results to the corresponding *Z*-acid. The results here show that this novel protocol is a tool of choice for the direct, rapid and sensitive detection of neutral and sulfated carbohydrates without any tedious *Z*-cinnamic acid preparation and isolation.

## Introduction

Since their introduction as matrices at the beginning of matrix-assisted UV laser desorption/ionization mass spectrometry (MALDI-MS) development, cinnamic acid derivatives, particularly 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid; SA)[1] and α-cyano-4-hydroxycinnamic acid[2] have been extensively used especially for protein and peptide analysis. Since these early times other commercial available cinnamic acids have been used for proteins[1] and oligodeoxyribonucleotides.[3] Efforts to prepare new compounds rationally designed to keep the cinnamic basic structure have been made too.[4,5] Cinnamic acids can exist in *E*- and *Z*- geometric forms (Scheme 1).[6] Both exist in nature (plants).[7] It is important to point out that the cinnamic acids used as matrices are the geometric *E* isomers (*E*-cinnamic acids) and although sometimes this fact is not specified it is an important point to take into account.

As is known classic MALDI cinnamic acid matrices (*i.e.*, *E*-SA;[8,9] *E*-3-methoxy-4-hydroxycinnamic acid and *E*-ferulic acid (*E*-FA)[8]) do not perform well in carbohydrate analysis.[9–15] Recently we prepared *Z*-cinnamic acids[16,17] and we studied their behavior as MALDI matrices for carbohydrate analysis and compared it with that of the corresponding *E*-isomer. This was the first attempt to check *Z*-isomers as matrices.[18] The results were quite good for neutral (positive ion mode) and especially for sulfated carbohydrate (negative ion mode) analytes. We




Scheme 1 Molecular structure of the two components of the MALDI matrix obtained by UV-irradiation of *E*-cinnamic acids in MeOH solution [*i.e.*, components of the matrix prepared by the irradiation of an *E*-SA MeOH solution called the irradiated *E*-SA matrix (I-*E*-SA): (a) *E*-SA, (b) *Z*-SA].

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focused our attention on carbohydrates because we were interested in the development of new matrices for sugar analysis.[19–22]

The family of compounds currently used as carbohydrate MALDI matrices includes materials with quite different structures such as: crystalline organic compounds (substituted acetophenones,[10] *i.e.*, 2,4,6-trihydroxyacetophenone and 2,5-dihydroxyacetophenone; benzoic acids,[10] *i.e.*, 2,5-dihydroxybenzoic acid (DHBA)), ionic liquid compounds[13–15] (*i.e.*, DHBA + butylamine and α-cyano-4-hydroxycinnamic acid (CHCA) + butylamine) and nanomaterials[15] such as carbon nanotubes (CNT)[15,21] and nanoparticles (NPs)[22,23] (*i.e.*, diamond and titanium oxide ($TiO_2$)).

The nature of a matrix and the method of sample preparation are still nowadays of critical importance for obtaining strong signals from carbohydrates because some compounds, which act as very effective matrices for some carbohydrate types, are ineffective for others.[10–15,23] This fact occurs for members within the same family of carbohydrates (neutral, acidic, sulfated, basic, *etc.*). Furthermore, the inherent chemical structure of some families of sugar makes it more difficult to carry out MALDI-MS analysis because highly efficient source decomposition (ISD) takes place and intact molecular ions cannot be detected. However, many of the problems associated with the loss of fragments (*i.e.*, sialylated N-linked sugars with the loss of sialic acid (ISD), in source lactonization of sialic acids with the loss of $H_2O$ [M − 18], *etc.*) can be overcome by a suitable and patient choice of matrix.[10–15,22] The MALDI-MS analysis of sulfated oligosaccharides is problematic because labile sulfate groups are frequently dissociated (ISD) and thus the ion species of intact molecules are hardly detected.[10–15,19,22] Among commercial cinnamics, *E*-SA has been checked for sulfated carbohydrates but the presence of the abundant matrix ions in the region of the molecular ion interfered with the detection.[9] Methods such as stabilization by the derivatization of sulfate groups[10–15] and the development of cool matrices such as 2,5-dihydroxybenzoic acid (gentisic acid, GA)[10] and ionic liquid matrices[15,24] have been reported. GA is a cool matrix widely used for carbohydrate analysis. A weak point of GA is the formation of inhomogeneous needle-shaped crystals, and therefore the analytes are ionized in only a few small areas on the probe called sweet spots. On the contrary, nor-harmane (9*H*-pyrido [3,4-*b*]indole, nHo) as a matrix provides abundant homogeneously distributed sweet spots all over the sample but behaves as a hotter matrix than GA in similar experiments.[25]

In the present paper we study and compare matrices of *E*- and *Z*-cinnamic acids with pre-prepared mixtures containing both *E*- and *Z*-acids with increasing amounts of the *Z*-form (Scheme 1). Quite good results were obtained with mixtures containing an *approx.* 1 : 1 mol $mol^{-1}$ *E*/*Z*-acid ratio (see Results and discussion). Thus taking into account these results we developed a quick and simple protocol for "photo-making at home" with the *approx.* 1 : 1 *E*/*Z* mixture from commercial *E*-cinnamic acids. The protocol is based on the *E*/*Z* photoisomerization of alkenes in solution.[26–30] The photochemical *E*/*Z* isomerization of alkenes is a well known one step process (Scheme S1 in ESI†).[29,30] It is a special tool used for synthesis, in preparative organic photochemistry.[26–28] It has a key role in many photobiological phenomena[26–30] and technological applications.[31,32] It has been described in the gas, liquid and solid states.[26–33]

Thus, by the *in situ* UV-irradiation of a methanolic solution of commercial *E*-cinnamics (*i.e.*, *E*-SA, *E*-FA, *E*-4-hydroxycinnamic acid (*E*-coumaric acid, *E*-CuA) and *E*-3,4-dihydroxycinnamic acid (*E*-caffeic acid, *E*-CAFA)) the corresponding *E*/*Z* mixtures were obtained. For the description of the results in the main text we focused our attention on *E*-SA, *Z*-SA, the pre-prepared *E*-SA + *Z*-SA mixtures and the irradiated *E*-SA methanolic solution (I-*E*-SA) (Fig. 1 and 2). The results obtained with FA, CuA and CAFA are herein briefly commented on and some of the results are shown in the ESI (Fig. S1–S5†).

The physical and spectroscopic properties of *E*-acid, *Z*-acid, the pre-prepared *E*-acid + *Z*-acid 1 : 1 (mol $mol^{-1}$) mixture, and the irradiated *E*-acid methanolic solution were compared; the irradiated *E*-acid methanolic solutions were characterized by $^1$H-NMR spectroscopy (see the ESI, Experimental and Fig. S6(a–d)†). The morphological properties of the solid samples prepared for the MALDI experiments with the *E*-acid, *Z*-acid and the irradiated *E*-acid methanolic solution containing an *approx.* *E*-acid + *Z*-acid 1 : 1 (mol $mol^{-1}$) mixture, were compared (*i.e.*, Fig. S7, ESI†). The corresponding Laser Desorption Ionization (LDI) mass spectra obtained were recorded for comparison too (Fig. S8 in ESI†). For the molecular modeling of the optimized geometry and stereochemistry of *E*-cinnamic and *Z*-cinnamic (*i.e.*, *E*-SA and *Z*-SA; molecular

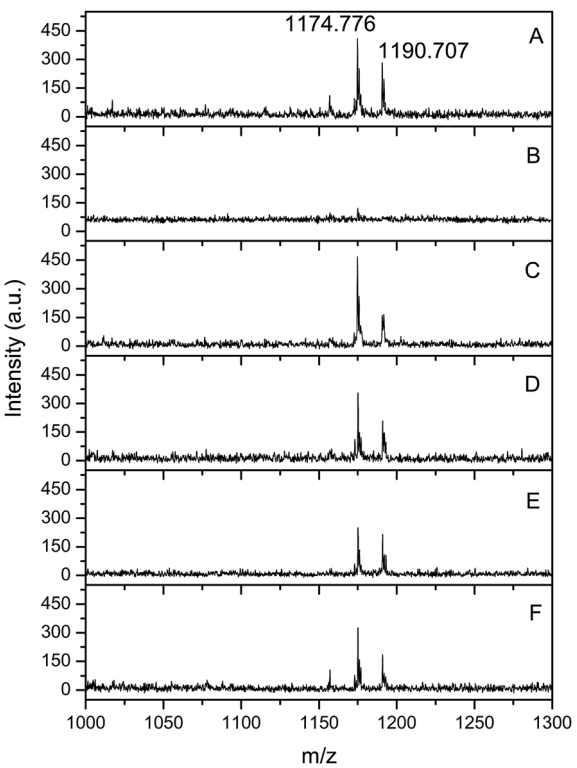


Fig. 1 The effect of irradiation of the *E*-SA methanolic solution on the performance of *E*-SA as a matrix. Positive ion mode. Analyte M7 ($M_w$ 1152.38) detected as $[M + Na]^+$ and $[M + K]^+$. Matrix: (A) *Z*-SA; (B) *E*-SA; (C) pre-prepared *Z*-SA + *E*-SA 1 : 1 (mol $mol^{-1}$) mixture; (D) I-*E*-SA 1 h; (E) I-*E*-SA 2 h; and (F) I-*E*-SA 3 h.

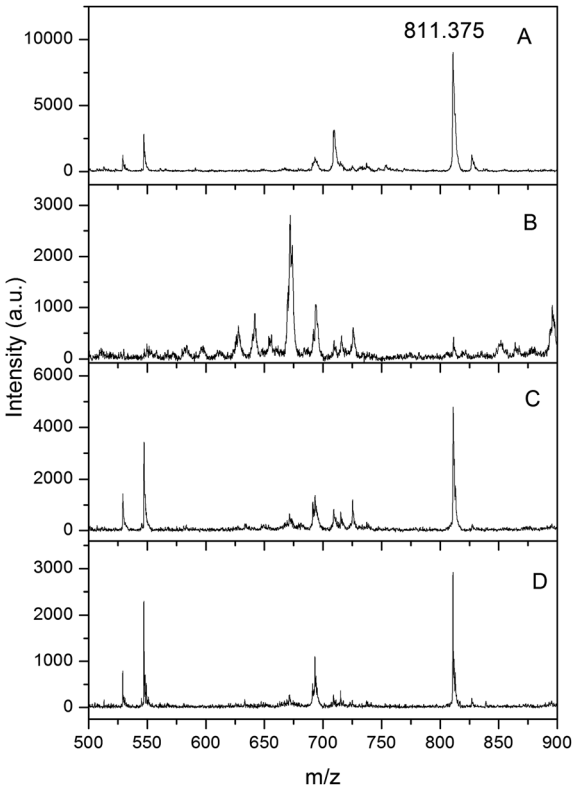


Fig. 2 The effect of irradiation of the *E*-SA methanolic solution on the performance of *E*-SA as a matrix. Negative ion mode. Analyte NCT ($M_w$ 834.62) detected as $[M - Na]^+$. Matrix: (A) *Z*-SA; (B) *E*-SA; (C) pre-prepared *Z*-SA + *E*-SA 1 : 1 (mol mol$^{-1}$) mixture; and (D) irradiated *E*-SA solution (I-*E*-SA) 3 h.

formula shown in Scheme 1 and S1†), density functional theory (DFT) at the unrestricted B3LYP/6-311G(d,p) was used.[34,35]

## Results and discussion

The *E*/*Z* photoisomerization of alkene compounds (Scheme S1, ESI†) has been described in different media.[7,16,17,26–33] In order to inspect this possibility for *E*-cinnamic acids in the solid analyte–matrix sample during MALDI mass spectrometry analysis, and to study its effect on the matrix behavior, different experiments were conducted. The intensity of the analyte signal of a selected carbohydrate (*i.e.*, b-CD as $[b\text{-CD} + Na]^+$) was monitored. The low intensity and poor resolution signal detected with *E*-SA did not improve after successive (100; 200; 300; 400; 500) shots at the same sweet spot position. Similar experiments conducted with *Z*-SA as the matrix showed that the higher intensity and better S/N ratio of the signals, compared with those obtained with *E*-SA, were kept after successive shots (results not shown).[18] On the contrary the behavior of *E*-SA as the matrix drastically improved when the pre-prepared mixtures of *E*-SA and *Z*-SA were used as the matrix. The solid sample was prepared as analyte + [*E*-SA + *Z*-SA], and obtained improved results when the *E*-SA + *Z*-SA mixture was enriched with the *Z* isomer. The best results required an *E*/*Z* ratio of *approx.* 1 : 1 (mol mol$^{-1}$). The trend in the S/N ratio for the analyte signals obtained was: *Z*-SA = *E*-SA + *Z*-SA 1 : 1 ⋙ *E*-SA. The results obtained by using maltoheptaose (M7) as the analyte are shown in Fig. 1 (Fig. 1, analyte: M7; matrix: (a) *Z*-SA, (b) *E*-SA; (c) pre-prepared *E*-SA + *Z*-SA 1 : 1 mol mol$^{-1}$).

As a conclusion, the *E*- to *Z*-photoisomerization was not occurring efficiently in the solid state during the MALDI experiment to increase the amount of *Z*-isomer in the solid sample remaining after the laser impact because the matrix behavior was not improved during the experiment. Furthermore, in order to improve the *E*-isomer performance as a matrix, *Z*-isomer should be added to the matrix used for sample preparation. Unfortunately *Z*-isomers are not commercially available and their preparation is not easy to conduct when lacking experience in organic synthesis. These facts clearly suggested that we should explore the *in situ* matrix solution photoisomerization of commercial *E*-cinnamics as a very simple and clean "one-pot" preparation of the *E*- + *Z*-cinamics mixture to be used as a matrix. The cinnamic acid *E*-/*Z*-photoisomerization in methanolic and in acetonitrile solutions efficiently yield *E*-/*Z*-mixtures with an *approx.* 1 : 1 mol mol$^{-1}$ ratio.[16]

In order to optimize the new protocol for matrix mixture photo-preparation, irradiations were conducted in a deuterated methanol solution for different time periods and we monitored the *E*- to *Z*-cinamic acid ratio obtained in the irradiated *E*-cinnamic acid solution by $^1$H-NMR. Simultaneously the behavior as a matrix of each irradiated solution was checked. The $^1$H NMR data for *E*-SA, *Z*-SA, irradiated *E*-SA (I-*E*-SA), *E*-FA, *Z*-FA, irradiated *E*-FA (I-*E*-FA), *E*-CuA, *Z*-CuA, irradiated *E*-CuA (I-*E*-CuA), *E*-CAFA, *Z*-CAFA and irradiated *E*-CAFA (I-*E*-CAFA) are listed in the ESI† section. Furthermore the corresponding spectra of I-*E*-FA, I-*E*-SA, I-*E*-CuA and I-*E*-CAFA, in which the diagnosis signals for quantitation are highlighted, are displayed in Fig. S6(a–d) (ESI†); these irradiated solutions showed optimum behavior as matrices.

As an example Fig. 1 shows the results obtained by using the matrices of I-*E*-SA obtained by UV irradiation for different time periods (*i.e.*, 1 h, 2 h, and 3 h) and M7 used as the analyte. The results obtained with b-CD as the analyte and matrices of *Z*-SA, *E*-SA, pre-prepared *E*-SA + *Z*-SA (1 : 1 mol mol$^{-1}$), I-*E*-SA 3 h irradiation and I-*E*-SA 8 h irradiation are shown in Fig. S9 (ESI†). Additional results obtained from the analysis of fructane (F5), maltopentaose (M5) and maltohexaose (M6) are also included as ESI (Fig. S10†). The results indicate that at *approx.* 1–3 h irradiation the *E*-SA methanolic solution reaches the photostationary equilibrium with an *E*/*Z* molar ratio of *approx.* 1 : 1. This ratio seems to be enough to drastically change the behavior of the *E*-isomer as a matrix. Fig. S1–S5 in the ESI† show additional results obtained with b-CD and M7 as analytes and *Z*-FA, *E*-FA, I-*E*-FA, *Z*-CuA, *E*-CuA, I-*E*-CuA, *Z*-CAFA, *E*-CAFA and I-*E*-CAFA as matrices. In all cases the results obtained support the conclusions obtained with SA.

The irradiated *E*-SA methanolic solution, as well as the irradiated methanolic solutions I-*E*-FA, I-*E*-CuA and I-*E*-CAFA, containing a mixture of *E*- and *Z*-species could be kept as stock solutions in a refrigerator and/or in dark conditions at room temperature for several weeks.

Sulfated oligosaccharide MALDI-MS analyses were conducted in both ion modes. The best results were obtained in

 

negative ion mode. The application of the *E*-SA, *Z*-SA, pre-prepared *E*-SA + *Z*-SA and I-*E*-SA matrices for sulfated oligosaccharides demonstrated that *E*-SA + *Z*-SA (1 : 1) and I-*E*-SA showed good performance in terms of sensitivity, limits of detection and the suppression of ISD of the sulfate groups as did *Z*-SA,[18] in negative ion mode. The performance of *E*-SA was the worst. When *E*-SA was used, the intact molecular ions as $[M - Na]^-$ and the corresponding main fragments were either not detected or seen just as very minor signals; abundant not assigned clusters were also observed (Fig. 2B). The fragmentation in negative ion mode mainly occurred by the dissociation of the sulfate groups corresponding to a loss of 102 Da from $[M - Na]^-$ to yield $[M - Na - nSO_3Na + nH]^-$ (*i.e.*, Frag. 1 = $[M - Na - SO_3Na + H]^-$; Frag. 2 = $[M - Na - 2SO_3Na + 2H]^-$; *etc.*).[10,24,25] Fig. 2 shows the spectra obtained in negative ion mode for neocarratetraose $4^1,4^3$-disulfate disodium salt (NCT; $M_w$ 834.62; the general molecular structure for sulfated carbohydrates is included in Scheme S2, ESI†) with *Z*-SA (Fig. 2A) and *E*-SA (Fig. 2B) as matrices. A significant suppression in the dissociation of the sulfate groups using *Z*-SA (Fig. 2A) and the importance of ISD when *E*-SA is the matrix can be observed (Fig. 2A; ions observed: $[M - Na]^-$ at *m*/*z* 811.375 as the main signal and $[M - Na - SO_3Na + H]^-$, Frag. 1, at *m*/*z* 709.61). The analyte signals were poorly detected when *E*-SA was the matrix (Fig. 2B). The additional figures which compare the spectra obtained for NCT using matrices of the pre-prepared mixture containing *Z*-SA and *E*-SA, (*Z*-SA + *E*-SA, 1 : 1 (mol $mol^{-1}$) (Fig. 2C)) and the 3 h irradiated methanol solution of *E*-SA (Fig. 2D, I-*E*-SA 3 h), which contains the photostationary mixture of *E*-SA + *Z*-SA (*approx*. 1 : 1 mol $mol^{-1}$), show that both spectra are very different to those obtained with *E*-SA and are quite similar to those obtained with *Z*-SA as the matrix.

As a conclusion of this section, the *E*-SA methanol solution after UV-irradiation (I-*E*-SA) behaved quite similarly to *Z*-SA in the analysis of sulfated sugars in negative ion mode and we can call it a "cold" matrix too (suppression of ISD). The additional advantage was that all over the sample prepared with I-*E*-SA, sweet spots were homogeneously distributed and were easy to find, similar to that observed with *Z*-SA. The optical images obtained for the morphological comparison of the surfaces of the solid samples prepared with NCT as the analyte and *Z*-SA, *E*-SA and I-*E*-SA as the matrices are included in the ESI (Fig. S7†). The solid samples prepared with I-*E*-SA looked more similar to *E*-SA than to *Z*-SA although the MALDI results were similar to those obtained using *Z*-SA as the matrix.

By using molecular modeling, special attention was paid to the stereochemistry of *Z*- and *E*-cinnamic acids. The optimized geometry for *E*-cinnamic acid showed a preferential almost flat and rigid molecule as is shown in Scheme 1(a) for *E*-SA. On the contrary, *Z*-cinnamic acid showed that the carboxylic acid group rotates and approaches the polar substituents located at C-3 and C-4 of the aryl group in a synclinal overlapping fashion to create a cavity limited by the polar substituents and the carboxylic group (*i.e.*, *E*-SA in Scheme 1(b)). The cavity provides the required distance among the functional groups located at the entrance, to allow the inclusion of a monosaccharide molecule and the generation of stabilizing intermolecular interactions such as hydrogen bonds with it. This approach and the interactions can easily occur with a small monosaccharide such as hexose and/or pentose, *i.e.*, glucose, and with any of the monosaccharide units included in an oligosaccharide such as β-cyclodextrin (β-CD) and higher $M_w$ oligosaccharides and polysaccharides. This would be the first level of control of the matrix–analyte molecular interactions in a solid sample when *Z*-SA is used as the matrix. There would be a selective interaction of *Z*-SA with a carbohydrate basic structure thanks to its peculiar stereochemistry. This special interaction at the crystal molecular level is not possible with *E*-SA because of its planar semi-rigid structure. A second level of control and differentiation of the process could be imparted through the higher efficiency of desorption (ablation) showed by *Z*-SA and its lower mp.

# Experimental

### Chemicals and materials

9*H*-Pyrido[3,4-*b*]indole (nor-harmane, nHo), 2,5-dihydroxybenzoic acid (DHBA; gentisic acid, GA), 2,4,6-trihydroxyacetophenone (THAP), *E*-4-hydroxycinnamic acid (*E*-coumaric acid, *E*-CuA), *E*-3-methoxy-4-hydroxycinnamic acid (*E*-ferulic acid, *E*-FA), *E*-3,5-dimethoxy-4-hydroxycinnamic acid (*E*-sinapinic acid, *E*-SA), *E*-3,4-dihydroxycinnamic acid (*E*-caffeic acid, *E*-CAFA) and aliphatic organic amines (ethanolamine, butylamine) were purchased from Aldrich Chemical Co. The sulfated neocarrabiose oligosaccharides (neocarratetraose $4^1,4^3$-disulfate disodium salt [NCT], neocarrahexaose $4^1,4^3,4^5$-trisulfate trisodium salt [NCH] and neocarraoctaose $4^1,4^3,4^5,4^7$-tetrasulfate tetrasodium salt [NCO]) were purchased from Sigma-Aldrich (USA). The cyclomaltoheptaose (β-cyclodextrin [b-CD]) and the maltoses (maltoheptaose [M7], maltohexaose [M6] and maltopentaose [M5]) were obtained from Sigma Chemical Co. Ltd, Tokyo, Japan. Fructans (fructose [F1], sucrose [F2], 1-kestose [F3], nystose [F4] and $1^F$-fructofuranosylnystose [F5]) were obtained from Wako Pure Chemical Industries, Japan. Representative molecular structures of the analytes used are shown in Scheme S2 (ESI†). All the solvents (Sigma-Aldrich HPLC grade), were used as purchased without further purification. Water of very low conductivity (Milli-Q grade) was used. *Z*-4-Hydroxycinnamic acid (*Z*-coumaric acid, *Z*-CuA), *Z*-3-methoxy-4-hydroxycinnamic acid (*Z*-ferulic acid, *Z*-FA), *Z*-3,5-dimethoxy-4-hydroxycinnamic acid (*Z*-sinapinic acid, *Z*-SA) and *Z*-3,4-dihydroxycinnamic acid (*Z*-caffeic acid, *Z*-CAFA) were synthesized as described elsewhere.[16] They were fully characterized (mp, $^1$H and $^{13}$C-NMR, UV-vis absorption spectroscopy; EI-HRMS) by comparison with the authentic samples previously described[16] (the $^1$H-NMR data for the *Z*- and *E*-acids are included in the ESI†).

### Sample preparation

The matrix stock solutions were made by dissolving 2 mg of the selected compound in 1 mL of methanol/water (65 : 35 v/v). The analyte solutions were freshly prepared by dissolving the carbohydrates (1 mg) in water (1 mL). To prepare the analyte–matrix sample the thin-film layer method[36] (sandwich method; method A), and the mixture method (method B) were used.

Method A: typically 0.5 μL of the matrix solution was placed on the sample probe tip and was air-dried at room temperature. Subsequently, 0.5 μL of the analyte solution was placed on the sample probe tip covering the matrix and partially dissolving it, and was air-dried. Then, two additional portions (0.5 μL) of the matrix solution were deposited on the same sample probe tip and were air-dried. The matrix to analyte ratio was 3 : 1 (v/v) and the matrix and analyte solution loading sequence was: (i) matrix, (ii) analyte, (iii) matrix, and (iv) matrix. Comparative experiments were also conducted with analyte–matrix samples prepared by the mixture method (method B). The pre-prepared mixture was obtained by mixing the matrix and analyte solutions in a 3 : 1 (v/v) ratio. Two portions (0.5 μL) of the mixture were successively loaded on the probe and were air-dried at room temperature. Similar results were obtained with both sample preparation methods.

### Photo-preparation of the matrix: irradiation of *E*-cinnamics in methanolic solution

The irradiation of *E*-SA in a methanol solution was carried out with UVB lamps. The lamp emitted in the wavelength range of 290 to 310 nm, with an emission maximum at 300 nm (Rayonet RPR lamp 300 nm, bandwidth ~20 nm, 8 W, 26 cm length; Southern N.E. Ultraviolet Co.). A solution of *E*-SA (15 mg) in methanol (5 mL) was irradiated in a Pyrex container placed at a distance of 7.5 cm from the lamp, under normal atmospheric conditions and magnetic stirring. To adjust the experimental conditions six solutions were irradiated for different times with one, two and three lamps, keeping the Pyrex container distance to each lamp constant (7.5 cm; geometrical distribution lamps and container as a static merry-go-round). The progress of the photo-reaction was monitored by $^1$H-NMR spectroscopy; in these cases deuterated methanol was used ($CD_3OD$). The photoisomerization progress of *E*-acid was as follows (average values): (i) one lamp, 5 min, 0% *Z*-acid; 10 min, 4.7% *Z*-acid, (ii) two lamps, 5 min, 1% *Z*-acid; 10 min, 9.9% *Z*-acid, (iii) three lamps, 3 h, 52% *Z*-acid; 4 h, 53% *Z*-acid; 8 h, 52% *Z*-acid, showing that in these experiments the photo-stationary state was reached (*E*/*Z* ratio *approx.* 1 : 1 (mol $mol^{-1}$)). Thus, an irradiation time of 3–4 h and 3 lamps were the best conditions to get a mixture of *E*/*Z* with *approx*. 1 : 1 mol $mol^{-1}$ (*i.e.*, irradiated *E*-SA solution, I-*E*-SA) (Fig. 1 and $^1$H-NMR data in the ESI, Fig. S6c†). The matrix stock solutions were made by adding 0.35 mL of water into 0.65 mL of the irradiated *E*-acid solution (65 : 35 v/v). A similar protocol was used for the preparation of I-*E*-FA, I-*E*-CuA and I-*E*-CAFA (ESI, $^1$H-NMR data and Fig. S6a, b and d†). To prepare the analyte–matrix sample the above described thin-film layer method (sandwich method, method A) and the mixture method (method B) were used.

### MALDI mass spectrometry experiments

Spectra were recorded on a Bruker Ultraflex II TOF/TOF, controlled by the FlexControl 3.0 software (BrukerDaltonics, Bremen, Germany). Desorption/ionization was performed using a frequency tripled Nd:YAG laser emitting at 355 nm with a 100 Hz shot frequency. All mass spectra were taken in the positive- and negative-ion modes and in the linear mode. Experiments were performed by using firstly the full range setting for laser firing position in order to select the optimal position for data collection, and secondly by fixing the laser firing position in the sample sweet spots. The laser power was adjusted to obtain a high signal-to-noise ratio (S/N) while ensuring minimal fragmentation of the parent ions and each mass spectrum was generated by averaging 200 laser pulses per spot. Spectra were obtained and analyzed with the programs FlexControl and FlexAnalysis, respectively. A MTP 384 target plate steel TF was used (Part no.: 209519; target frame (# 74115); 384 circular spots, 3.5 mm diameter; S/N 03 630).

### Molecular modeling

The ground state geometry of the *Z*- and *E*-cinnamic acids was fully optimized without imposing any symmetry constraints by *ab initio* and semiempirical methods (Scheme 1, (a) *E*-SA and (b) *Z*- SA; see details included in the ESI†).[34,35]

## Conclusions

To conduct our study of the application of irradiated solutions of *E*-cinnamic acids containing a mixture of *E*- + *Z*-forms as MALDI matrices for carbohydrates, their performances as matrices for commercially available fructans, maltoses, cyclodextrins and sulfated sugars were investigated. Experiments were conducted in positive and negative ion modes. The best results were obtained in the former for neutral sugars and in the later for sulfated sugars. As was demonstrated the efficiency of the desorption/ionization of each carbohydrate as a positive ion (*i.e.*, $[M + Na]^+$ and/or $[M + K]^+$), and negative ion (*i.e.*, $[M - Na]^-$) as well as the cluster formation depend on the presence of *Z*-acid in the matrix. Although *Z*-SA was shown to be better than *E*-SA, its performance is quite similar to that of the *E*-SA + *Z*-SA mixture (*approx.* 1 : 1 mol $mol^{-1}$) obtained by the UVB irradiation of a *E*-SA methanolic solution (photoisomerization). When the behavior of the other cinnamic acids studied (CUA, FA and CAFA) was compared, when used as a matrix, the UVB irradiated methanolic solutions of the *E*-forms containing the *E*-form and the photo-generated *Z*- form (*E*- + *Z*-), similar conclusions were obtained.

In general SA performed better as a matrix for carbohydrate analysis than the other cinnamic acids studied although in each case the mentioned trend of behavior, $Z = E + Z \ggg E$, was always observed.

Molecular modeling, as an additional tool, showed that as a consequence of a geometry change in the rigid alkene bond of the cinnamic moiety, the stereochemistry of the matrix molecule changed dramatically and that at the molecular level the analyte–matrix interaction did too.

Taking into account the increasing interest in MALDI mass spectrometry application in the field of carbohydrate structural analysis,[23] here we propose an additional tool made-at-home as an extra possibility at the moment of choosing a suitable matrix for solving the problem of analysis of some not friendly carbohydrates.

 

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