Electronic Supplementary Material (ESI) for Chemical Communications.

SUPPLEMENTARY MATERIAL

Boronic acid chemistry in MALDI MS: a step forward in designing a reactive matrix with molecular recognition capabilities

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EXPERIMENTAL

Materials

4-formyl-phenylboronic acid (FPhB), cyanoacetic acid, α-cyano-4-hydroxycinnamic acid (CHCA), glycerol, 1,3- and 1,2-propandiol, 1,2-ethandiol, 1,3-butandiol, 1,4-butendiol, triethylenglycol, 1,2-pinandiol, norepinephrine, glycolic acid, mandelic acid, were obtained from Sigma-Aldrich (Sigma Aldrich, St. Louis, MO, USA). 9-Aminoacridine hemihydrate (9AA) was purchased from Acros Organics (Morris Plains, NJ). Water, acetonitrile (ACN) and methanol (MeOH), HPLC grade, were obtained from Sigma-Aldrich. Other materials (analytical grade) were commercially available. Human urines were collected from healthy donors.

Synthesis of CCPBA

CCPBA was synthesized according to a standard Knoevenagel condensation using cyanoacetic acid and 4-formyl-phenylboronic acid. Ammonium acetate was used as a catalyst. Two grams of cyanoacetic acid (1 equiv), 0.9 equiv of 4-formyl-phenylboronic acid, and 0.15 equiv of ammonium acetate were refluxed under stirring in sufficient amounts of toluene (ca. 50 mL). After quantitative separation of the reaction water by a Dean−Stark apparatus (ca. 3 h), the reaction mixture was cooled to 50°C and filtered. The crude product was washed with sufficient amounts of distilled water and purified by recrystallization from 70% acetonitrile/30% water performed according to standard protocols. ^{1}H NMR δ ppm: 7.90 (d, *J*= 8.0 Hz, 2H), 7.98 (d, *J*= 8.0 Hz, 2H), 8.26 (s, 1H); ^{13}C NMR δ ppm: 108.65, 117.95, 130.38, 134.84, 135.32, 153.65, 166.58. Anal. Calcd for $C_{10}H_8BNO_4$: C, 55.35; H, 3.72; N, 6.46. Found: C, 55.15; H, 3.69; N, 6.35.

Matrix characterization

UV-vis analysis. Spectra acquisition was performed using a UV/Vis/NIR Cary 5 Spectrophotometer (Varian – Palo Alto, CA, USA). A 0.046 M solution of each compound was prepared in ACN:MeOH (2:1 v/v). Then, the UV spectrum of a diluted solution (20 μM) was acquired in the 250-500 nm range.

LDI analysis. A 0.046 M solution of each compound was prepared in ACN:MeOH (2:1 v/v) and 1 μL was spotted directly on the target plate. The LDI-ToF MS spectrum was acquired both in positive and negative ion mode (see below).

FT-IR spectroscopy. The FT-IR spectroscopy investigation was performed using a PerkinElmer 1600 FT-IR spectrometer (PerkinElmer, Italy). FT-IR spectra were recorded on CHCA, FPhB and CCPBA mixed with an appropriate amount of KBr to obtain a final concentration of the sample of 1% w/w. The range examined was 4,000–400 cm^{-1} with a resolution of 1 cm^{-1}.

MALDI-TOF MS

MS experiments were performed using a Micromass M@LDI™ - LR time-of-flight mass spectrometer (Waters MS Technologies, Manchester, UK) equipped with a nitrogen UV laser (337 nm wavelength), reflectron optics and a fast dual micro-channel plate (MCP) detector.

Negative ion spectra were acquired in reflectron mode. The following voltages were applied: pulse, 2640 V; source, 15000 V; reflectron, 2000 V; MCP 1900 V; supply, 5000 V. The laser firing rate was 5 Hz, and, unless otherwise specified, 40 laser shots, obtained by a random rastering pattern, were used for each well. The resulting spectra were averaged, background subtracted, and smoothed by a Savitzky-Golay algorithm.

Samples preparation

After different solubility tests, the matrix stock solutions (10 mg/mL or 0.046 M) were prepared in ACN:MeOH (2:1, v/v ratio). Various model analytes were prepared at 1 mg/mL in MeOH. Then, 50 µL of each analyte solution were mixed with 50 µL (1:1, v/v ratio) and incubated for 20 min at room temperature. For these experiments, matrix/analyte ratios ranging from 5000 to 1 were examined and the best results were obtained with matrix/analyte ratio in the range 3-10. 1 µL of the analyte-matrix solution was spotted directly on the target plate and analysed by MALDI-TOF-MS. For urine analysis, the samples were dilute 10 times with water and incubated with CCPBA for 20 minutes. Then, 1 µL of the solution was spotted directly on the target plate and analysed by MALDI- MS.

Analyte Mass Calculation

For identification of analytes, simple rules can be followed. Firstly, the analyte should bear functionalities than can react with boronic acids (see ref 14 in the main text) as vic-diols, α-hydroxyacids or aminols to condense with the matrix.

Thus, the mass M_u of the unknown compound (in its neutral form) can be calculated as follows:

$M_u=M_{ex}-nM_m+2nM_{H2O}+1$ (usually n=1 or 2)

where M_{ex} is the experimentally measured mass of the deprotonated molecular ion, M_m is the mass of the CCPBA matrix (neutral form), M_{H2O} is the mass of water and n (typically 1 or 2) is the stoichiometric coefficient.

Moreover, a further validation is done comparing the experimental isotopic pattern with the theoretical one (for a given value n) as shown, for instance, in Fig.4 in the main text.

Sensitivity test

Herein, 2,3-butandiol was selected as representative to test the detection performances of CCPBA to *vic*-diols giving a minimum value of 50 pmol/spot. To this aim, two different experiments were performed: in the first one a stock analyte solution (10 nmol/µL) was successively diluted and then

mixed with CCPBA at a fixed concentration (30 nmol/μL); alternatively, the stock 2,3-butandiol solution (10 nmol/μL) was first mixed with CCPBA (30 nmol/μL) and then diluted at various concentrations. The MALDI analyses showed that the sensitivity was better in the latter case reaching a minimum value of 50 pmol/spot. This finding can be explained by assuming that improved performances can be obtained when the optimal matrix/analyte ratio 3:1 is retained.

Safety Considerations

Boronic acids present no particular environmental threat, and the ultimate fate of all boronic acids in air and aqueous media is their slow oxidation into boric acid which is relatively harmless, and may be toxic only under high daily doses[1]. As evidenced by some applications in medicine, most boronic acids present no particular toxicity[2] and small water-soluble boronic acids demonstrate low toxicity levels, and are excreted largely unchanged by the kidney[3].

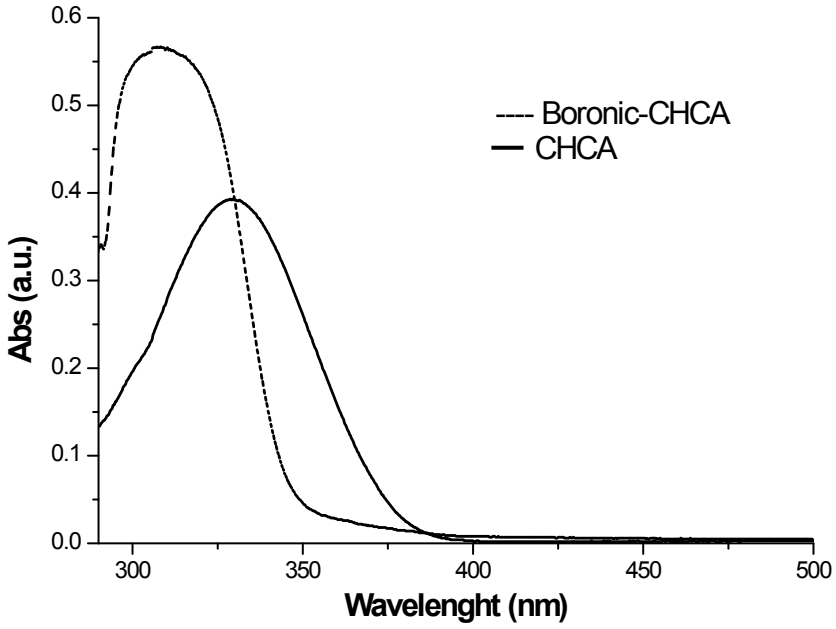

Figure S1. UV-vis spectra relevant to boronic-CHCA (CCPBA, dotted line) and CHCA (solid line).

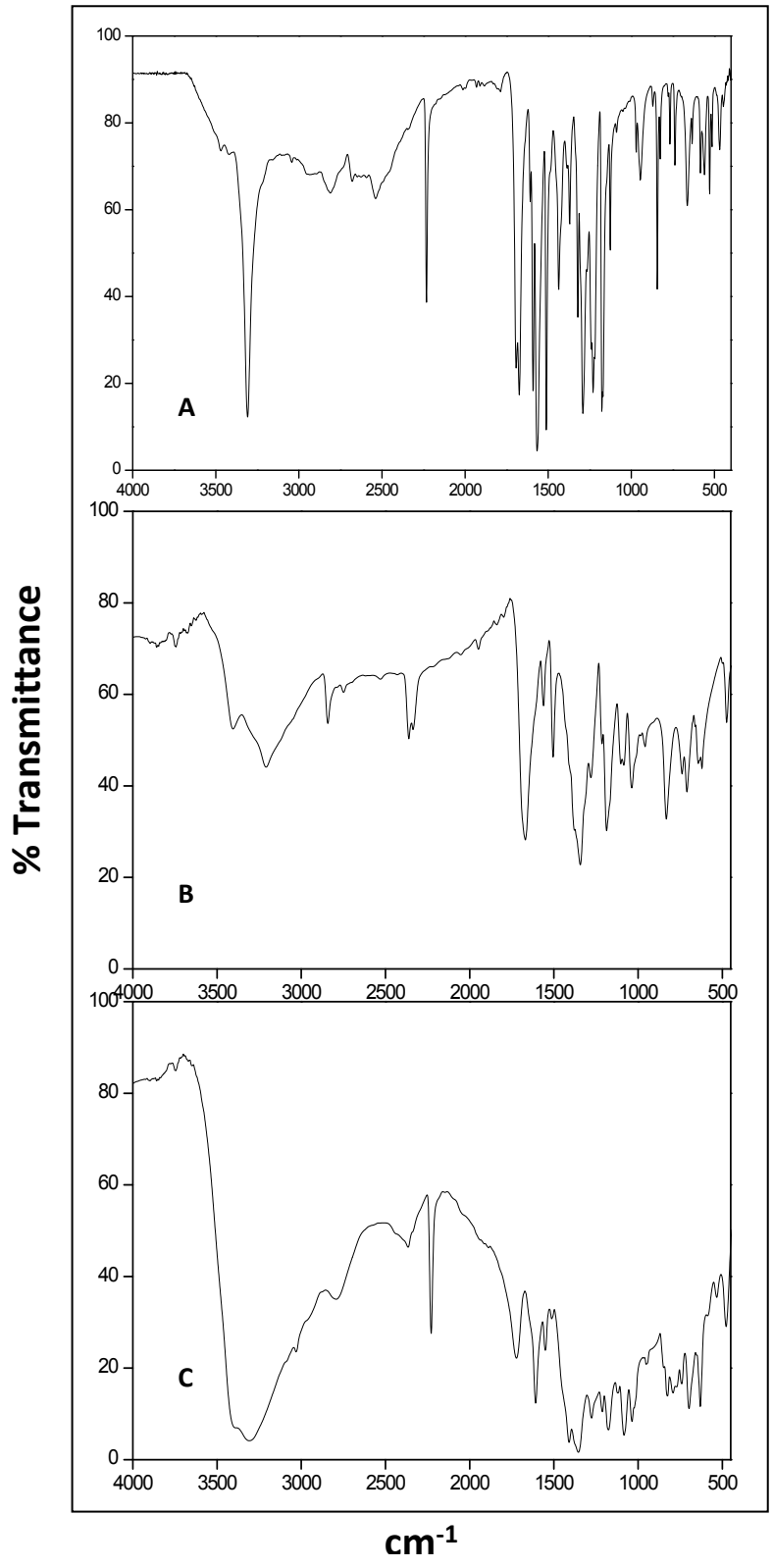

Figure S2. Figures S2(A), (B) and (C) show the FTIR spectra relevant to CHCA, 4-formyl-phenylboronic acid (FPhB) and CCPBA (**1**), respectively. The IR spectra in Fig. S2(A) and (B) are similar to that reported in the literature[4] while a different profile was obtained for the compound **1**. In particular, the peak at 2230 cm^{-1} characteristic of the CN stretch of a nitrile group (present also in CHCA, Fig S2A) and the broad peak at 1376 cm^{-1} associated with the C–B vibrations (present also in FPhB, Fig S2B) confirm the successful reaction.

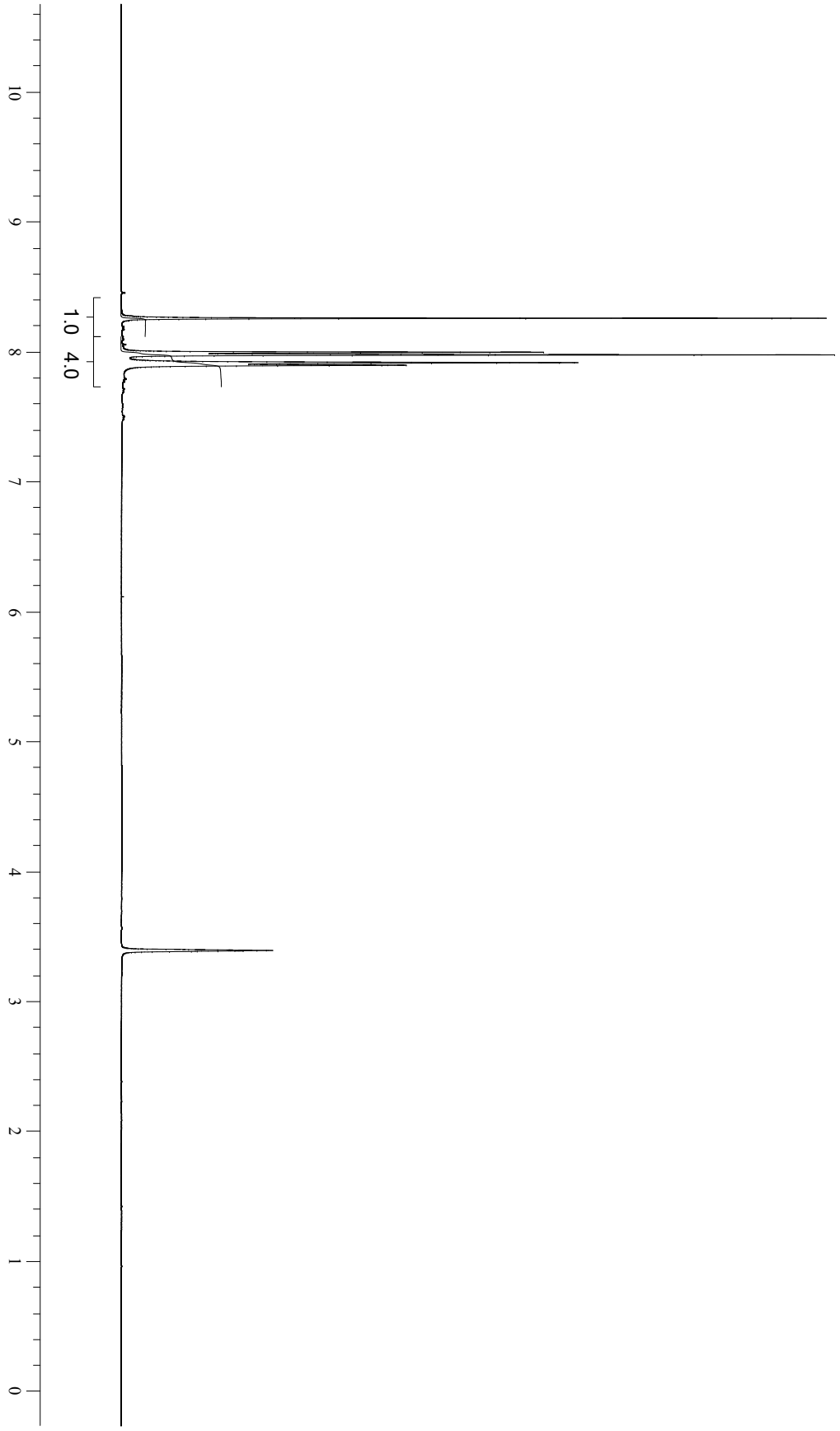

Figure S3. ^{1}H NMR spectrum of CCPBA.

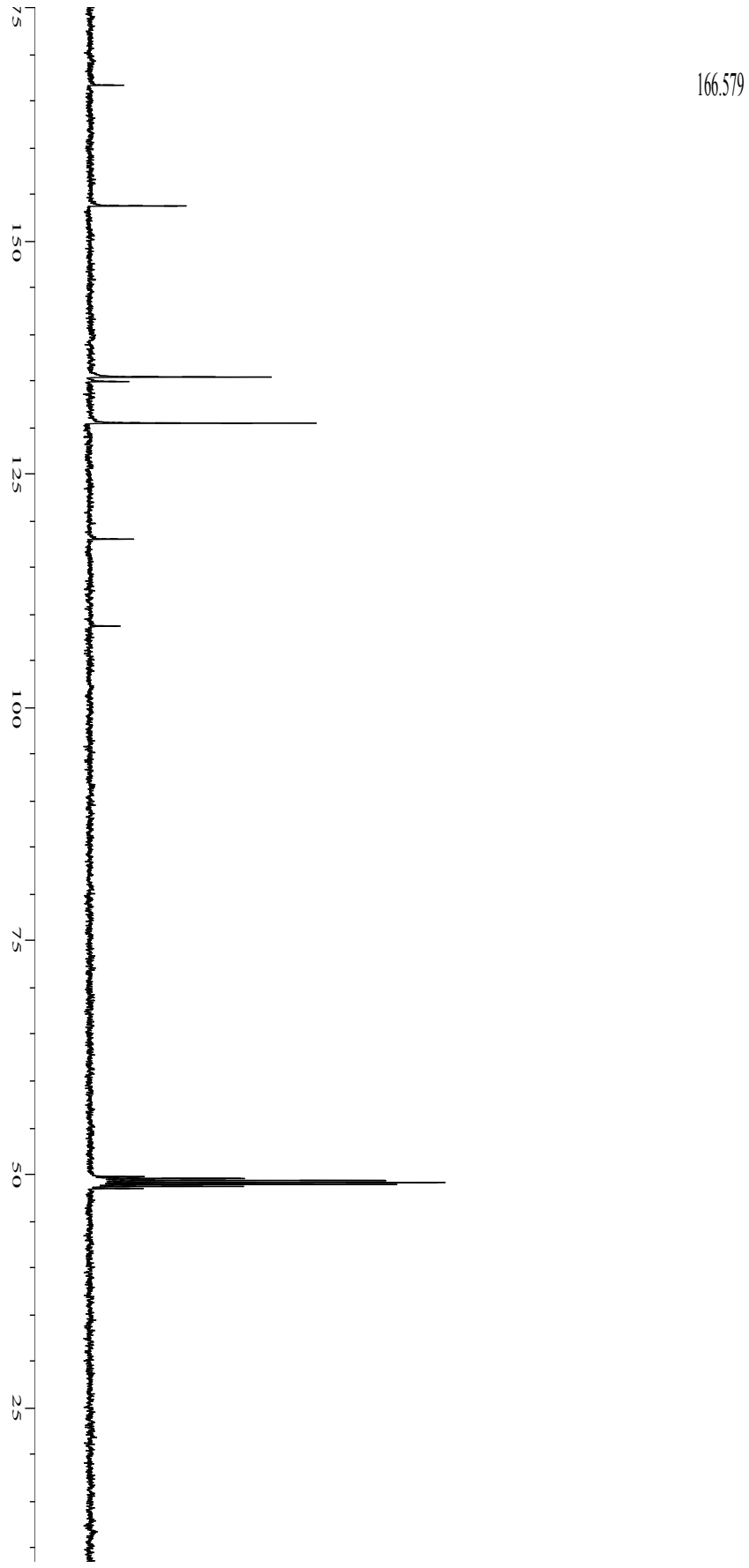

Figure S4. ^{13}C NMR spectrum of CCPBA.

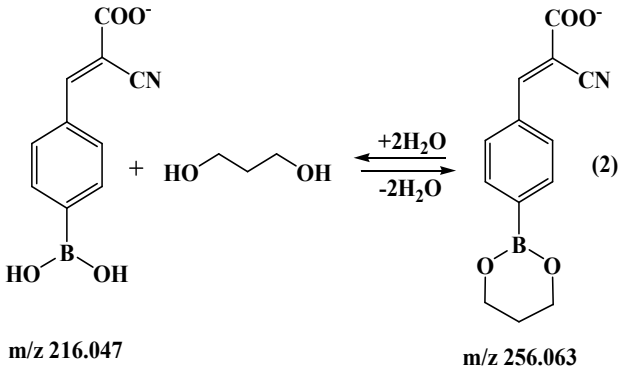

Figure S5. Scheme of the reaction between CCPBA and 1,3-propandiol.

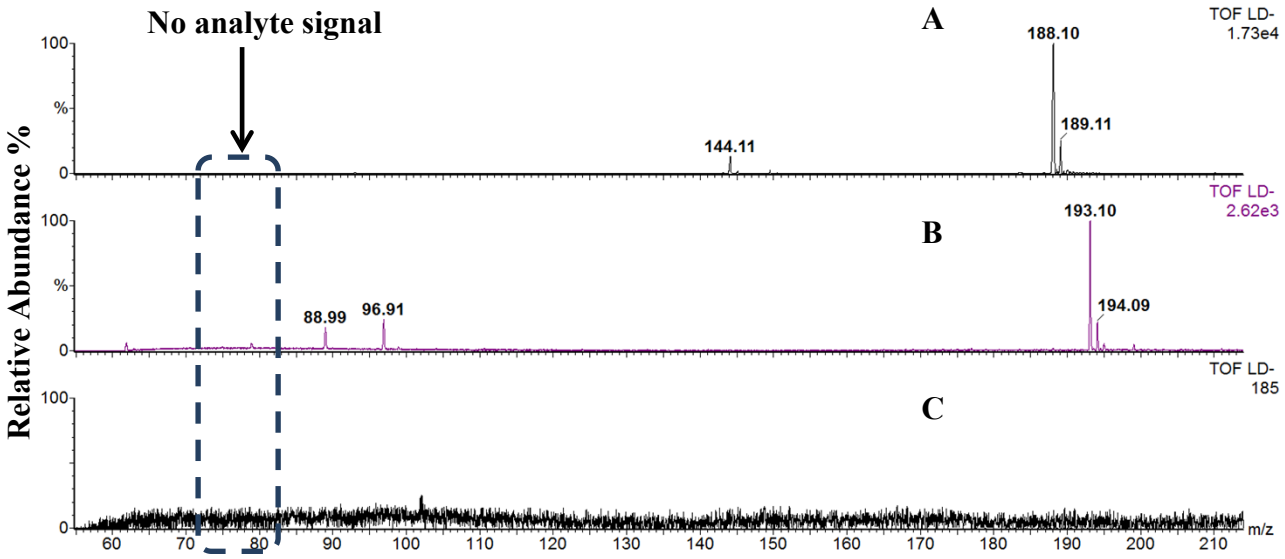

Figure S6. MALDI spectra relevant to 1,3-propandiol analyzed with CHCA (A), 9-aminoacridine (B) and 4-formyl-phenylboronic acid (C) as matrices.

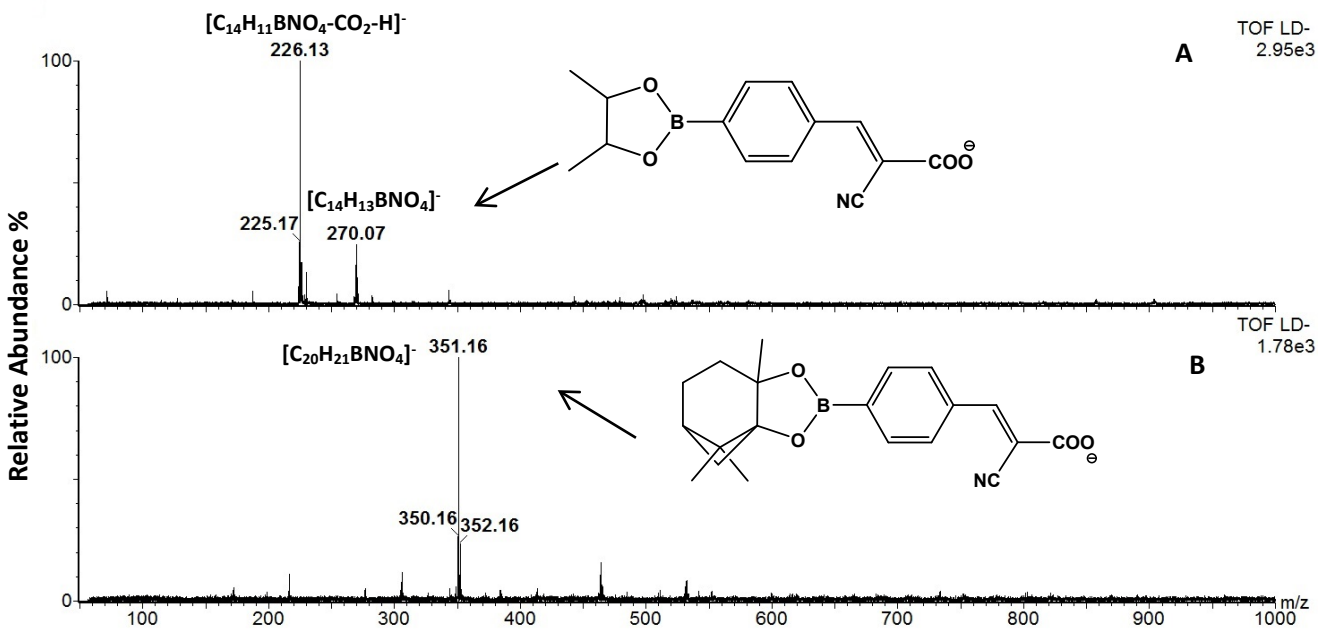

Figure S7. *Re*MALDI spectra relevant to 2,3-butandiol (A) and 1,2-pinandiol (B) reacted with CCPBA.

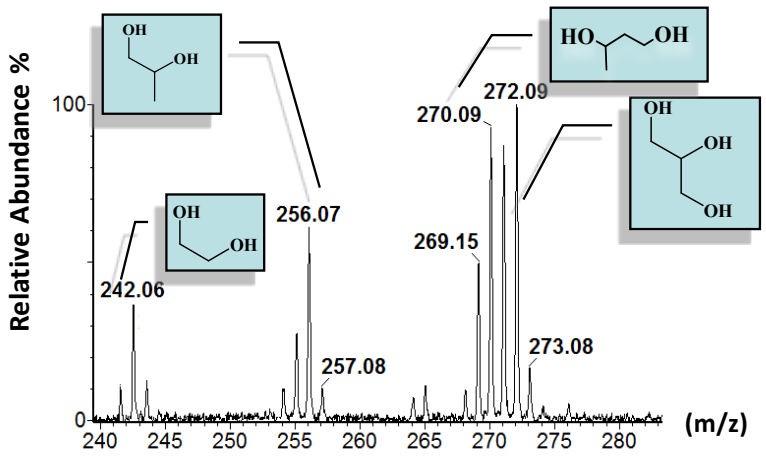

Fig. S8. *Re*MALDI mass spectrum showing the ionic species arising from the reaction between CCPBA matrix and various diols.

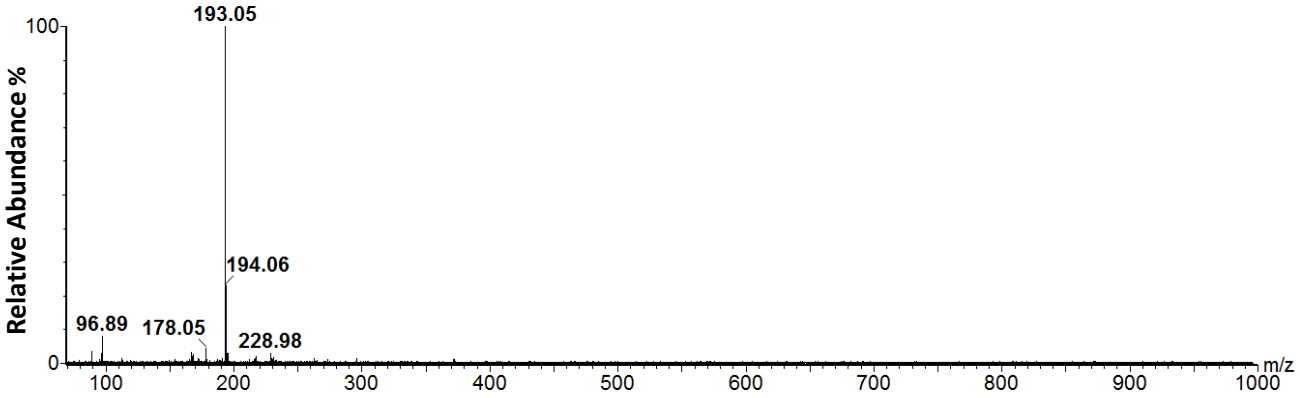

Figure S9. MALDI spectrum relevant to human urine mixed with 9-aminoacridine as a matrix.

Table S1. Major metabolites from human urine reacted with CCPBA (tentative attributions).

no	Observed m/z	Theorethical m/z*	Probable attribution
1	283.11	283.09	β-aminoisobutyric acid
2	327.09	327.11	carnitine
3	353.09	353.14	D-xylose $[M-2H+Na]^-$
4	365.02	365.03	L-Phosphoserine
5	372.00	372.06	Citric acid
6	437.09	437.12	5-methylcytidine/3-methylcytidine
7	438.09	438.11	3-methyluridine
8	463.09	463.12 463.12	Guanosine Isoguanosine
9	464.09	464.10	Xanthosine
10	480.07	480.09	Urate-3-ribonucleoside
11	481.08	481.11	5-carbamoylmethyluridine
12	482.12	482.18	6-ketoestriol
13	505.11	505.14	N2 N2 7-trimethylguanosine
14	507.12	507.20	1-ribosyl-N-ϖ-valerylhistamine
15	526.18	526.23	Cortexolone
16	592.12	592.14	N6-threonylcarbamoyladenosine
17	636.19	636.21	ε-(gamma-Glutamyl)-lysine (+2 boronic acid)
18	661.15	661.13	Urate-3-ribonucleoside (+2 boronic acid)
19	662.16	662.15	5-carbamoylmethyluridine (+2 boronic acid)

*rounded to the second decimal place

References

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2. M. Benderdour, T. Bui-Van, A. Dicko, et al., *J. Trace Elem. Med. Biol.* 1998, **12**, 2–7.
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4. (a)C.D. Calvano, S. Carulli and F. Palmisano, *Rapid Comm. Mass Spectrom*., 2009, **23**, 1659-68; (b) http://sdbs.db.aist.go.jp/sdbs/cgi-bin/cre_index.cgi